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Iohexol gastrointestinal transit times in cockatiels under sedation with intranasal midazolam by computed radiography

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ABSTRACT: *This study used contrast radiography to evaluate gastrointestinal transit times in cockatiels (Nymphicus hollandicus) and investigated the sedative effects of intranasal midazolam in this species and its usefulness in facilitating the manual restraint required for radiographic studies. Twelve healthy adult cockatiels received intranasal midazolam at dose of 2 mg/kg, and iohexol at 15 ml/kg by crop gavage. Radiographic images were obtained before contrast administration, 3 minutes after and then each 10 minutes for 90 minutes. Sedation quality of the bird was evaluated during the radiographic study and assessed according to an adapted visual sedation scale. Three minutes after iohexol administration, the cervical oesophagus and the crop were filled in all birds. At the same time, the contrast medium reached the thoracic oesophagus, proventriculus, isthmus and ventriculus in most birds. In all cockatiels, median (range) transit times were 3 (3-10) minutes for proventriculus and ventriculus, 10 (10-40) minutes for small intestine and 45 (30-70) minutes for large intestine. The overall gastrointestinal transit time was 50 (30-90) minutes.Crop remained filled with iohexol throughout the study, while oesophagus and isthmus presented a pattern of contrast progression different from the other gastrointestinal segments. According to the visual sedation scale, cockatiels presented a moderate to intense muscular relaxation, and intranasal midazolam seems to be an appropriate sedation protocol for radiographic study. All cockatiels remained healthy after the study and presented clear and watery stools at least 12 hours after, due to gastrointestinal emptying.*
Key words: *birds, imaging diagnosis, x-ray, iodine, Nymphicus hollandicus.*

Tempos de trânsito gastrintestinal com iohexol em calopsitas, sob sedação com midazolam intranasal, por meio de radiografia computadorizada

RESUMO: *O presente estudo objetivou determinar os tempos de trânsito gastrintestinal de calopsitas (Nymphicus hollandicus) por meio do estudo radiográfico contrastado e investigar os efeitos sedativos do midazolam intranasal nesta espécie, bem como a viabilidade do uso deste fármaco para facilitar a contenção manual durante o exame radiográfico. Doze calopsitas, adultas e saudáveis, receberam midazolam intranasal, na dose de 2 mg/kg, e 15 ml/kg de iohexol por gavagem. Imagens radiográficas foram obtidas antes da administração do meio de contraste, três minutos depois da administração e a cada 10 minutos, até 90 minutos de estudo. A qualidade da sedação foi avaliada durante todo o estudo radiográfico por meio de escala visual adaptada. Três minutos após a administração do iohexol, esôfago cervical e inglúvio foram preenchidos em todas as aves. Ao mesmo tempo, o meio de contraste alcançou esôfago torácico, proventrículo, istmo e ventrículo na maioria dos animais. Em todas as aves, a mediana e intervalo dos tempos de trânsito foram três (3-10) minutos em proventrículo e ventrículo, 10 (10-40) minutos em intestino delgado e 45 (30-70) minutos em intestino grosso, sendo que o tempo total de trânsito gastrintestinal foi 50 (30-90) minutos. Inglúvio permaneceu preenchido por meio de contraste durante todo o estudo radiográfico, enquanto esôfago e istmo apresentaram padrão de trânsito diferente dos demais segmentos avaliados. As aves apresentaram moderado a intenso relaxamento muscular durante o estudo e a administração de midazolam intranasal mostrou-se como protocolo sedativo apropriado em calopsitas. Todas as aves permaneceram saudáveis e apresentaram fezes com aspecto aquoso e esbranquiçado no mínimo 12 horas após o estudo radiográfico, devido ao esvaziamento gastrintestinal.*
Palavras-chave: *aves, diagnóstico por imagem, raios-x, iodo, Nymphicus hollandicus.*

INTRODUCTION

The cockatiel (*Nymphicus hollandicus*) is a pet bird kept worldwide, increasing the need by owners for veterinary care. For this reason, there is a demand for improving diagnostic tools that are essential in guaranteeing disease treatment and improving the health and welfare of this species (ASSIS et al., 2016; GRESPAN & RASO, 2017).

Gastrointestinal (GI) diseases are common in birds (GRESPAN & RASO, 2017; NEMETH et al., 2016). Among the useful and accessible methods to diagnose these diseases, are the positive-contrast GI studies. Contrasted radiographic images can provide

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information about the localisation, size and shape of the GI organs. When obtained in series, it is possible to evaluate transit times, the filling and emptying of the GI organs, as well as assisting with identification of other disorders, such as foreign bodies, neoplasms and perforations (BEAUFRÈRE et al., 2010; DOSS et al. 2017a; ERNST et al., 1998; KUBIAK & FORBES, 2012; SILVA et al., 2012).

Iohexol is a nonionic iodinated contrast medium with low osmolality. These characteristics make it suitable and safe for use in birds, due to its short transit time and minimal adverse effects in comparison with other contrast media such as barium sulphate. A short transit time is an attractive property in the treatment of birds, because it can provide a rapid diagnosis with minimal stress due to handling (MARTEL et al., 2018; NAGUIB, 2017).

During radiographic studies, stress to the bird due to repeated manual restraint is an inevitable component (DOSS et al., 2017a, 2017b). Therefore, it is important to prevent stress responses as much as possible, considering all the physiological consequences observed in cockatiels (DOSS et al., 2018).

In birds, intranasal administration of midazolam is suggested as a sedation protocol to facilitate procedures that do not call for analgesia and require a short time of immobilization, such as radiographic examinations. In addition, midazolam is reported to minimize stress responses due to manual restraint (DOSS et al., 2018; MANS et al., 2012; MARTEL et al., 2018; SCHÄFFER et al., 2017; SCHÄFFER et al., 2016).

This study used positive-contrast radiography with iohexol as contrast medium in the evaluation of transit times of GI organs in cockatiels under sedation with intranasal midazolam. Also, investigated sedative effects of intranasal midazolam in a lower dose in cockatiels and its usefulness in facilitating the manual restraint required for radiographic studies.

MATERIALS AND METHODS

Twelve adult cockatiels (*Nymphicus hollandicus*) were collected from a commercial breeder in the city of Capanema – Paraná – Brazil and transferred to the UFFS Veterinary Hospital (SUHVU/UFFS) located at city of Realeza – Paraná – Brazil, where the study was performed.

Cockatiels were estimated to be approximately 1 year old according to the breeder, systemically healthy, with no history of previous diseases and of undetermined sex.

The health status of each bird was investigated by a complete physical examination of body condition, hydration status and heart and respiratory rates. None had clinical signs of GI disease at the time the research was performed (GRESPAN & RASO, 2017).

The animals were housed in an experimental room and remained there throughout the research period. Temperature and humidity of the room were measured daily by a digital thermohygrometer (Incoterm®, Brazil). Birds received 12 hours of artificial light (6 am–6 pm), controlled by a timer (Enerbras®, Brazil). Two birds were housed in each wire cage of 60 × 60 × 60 cm, containing wood perches, feeding bowls and toys (ASSIS et al., 2016).

Feed and water were provided *ad libitum*. Feed was based on a commercial seed mixture daily (Bepê Distribuidora de Produtos Agro veterinários LTDA, Brazil) and eggs and fruits sporadically.

The experiment took place between September and November. On radiographic study day, food was withheld at 10 am, which was 4 hours before the initiation of the study. Each bird was removed from the cage by hand using a towel and weight was obtained using a digital scale.

Then, with the bird manually restrained – which entailed gentle manual restraint with a towel that covered the dorsum of the animal, holding the head and the wings in position against the body – midazolam (5 mg/ml, Hipolabor, Brazil) was administrated as an intranasal dose of 2 mg/kg divided equally between the two nostrils, using a 1-ml syringe and a 24-gauge catheter – a similar procedure has previously been described in cockatiels, parrots and macaws (DOSS et al., 2018; MANS et al., 2012; SCHÄFFER et al., 2017; SCHÄFFER et al., 2016).

After midazolam administration, the bird was placed in a perforated plastic box of 40 × 25 × 15 cm (length × depth × height), remaining there for 10 minutes.

Then, the animal was removed from the box and positioned to obtain the first two radiographic images, in right lateral (RL) and ventrodorsal (VD) views, before contrast medium administration.

With the bird in upright position, iohexol (Omnipaque® 350, Iohexol 350 mg I/ml, GE Healthcare, China), without dilution, was administered slowly by crop gavage, within the crop, using a 3-ml syringe and a 16-gauge catheter, at a dose of 15 ml/kg. After holding the bird in upright position for 3 minutes, the sequential images were performed.

Radiographic images, in RL and VD, were obtained 3 minutes after iohexol administration and every 10 minutes, to complete 90 minutes of study.

To obtain the radiographic images, the bird was held in an acrylic homemade positioning device, as used by SILVERMAN & TELL (2010b), which was placed over the radiographic receiver plate. The device had a guillotine, which was applied to the bird's neck to hold the head, while the wings were restrained using two heated bags and the legs by cotton straps tied to the device. A towel covered the bird's head while in radiographic position and a gauze pillow was used to keep bird's head elevated.

The radiographic images were obtained using mobile x-ray equipment (model Magvet® 320 mA 125 kv), processor and digital press (CARESTREAM®, models DirectView® CR XE and DryView® 5950), with compatible software. The radiographic technique was standardized at 320 mA, 10 mAs, 45 kV and 0.03 seconds.

During intervals between radiographic images, the bird remained inside the plastic box, with no visual or sound stimuli, with a heat source.

Sedation quality of the bird was evaluated throughout the radiographic study and assessed according to an adapted visual sedation scale (SCHÄFFER et al., 2016; Table 1). At the time of positioning for radiographic study, a sedation score, from 1 to 4, was recorded for each bird, according to the criteria of table 1.

After the study, the bird was placed back in its cage with a heat source, food and water and remained under observation until it had completely recovered.

The following organs were evaluated in all radiographic images from each cockatiel, based on descriptions of SILVERMAN & TELL (2010a) and KÖNIG et al. (2016): cervical and thoracic oesophagus, crop, proventriculus, proventricular-ventricular isthmus, ventriculus, small intestine, large intestine and cloaca.

A descriptive analysis was performed of GI transit times and sedation scores. Overall transit times, in addition to proventriculus, ventriculus, small and large intestine transit times in separate, were determined in minutes, by means of median and range.

RESULTS

The mean body weight of the cockatiels on the day of radiographic study was 0.120± 0,009 kg. All birds remained clinically healthy throughout the study.

Before iohexol administration, ventricular content was observed in 9 of the 12 birds, even after 4 hours of fasting. The content appeared similar in all birds, as a mineral opaque content, consisted with grit (Figures 1A and 2A).

Three minutes after iohexol administration, the cervical oesophagus and crop were filled in all birds (Figures 1B and 2B). At the same time, the contrast medium reached the thoracic oesophagus, proventriculus, isthmus and ventriculus in most birds (Figures 1B and 2B).

In all birds, contrast medium highlighted proventriculus and ventriculus at 3 (3-10) minutes (Figures 1B and 2B), 10 (10-40) minutes for small intestine (Figures 1C -1H and 2C-2H) and 45 (30-70) minutes for large intestine (Figures 1I and 2I).

The overall GI transit time of the cockatiels, which was defined as the time to the

Table 1 - Sedation scores according to the behaviour of adult cockatiels (*Nymphicus hollandicus*) under sedation with intranasal midazolam at a dose of 2 mg/kg during physical restrain for radiographic study.

Scores	Behaviour
1	Uncooperative, absence of muscular relaxation, wing movements, vocalisation and attempting to peck. Needs physical restrain.
2	Light sedation. Moderate muscular relaxation, wing movements whenever handled, alert to external stimuli, attempting to peck without force. Needs light restrain.
3	Moderate sedation. Intense muscular relaxation, sporadic wing movements, alert to external stimuli, no pecking. No need for restrain.
4	Intense sedation. Intense muscular relaxation, no wing movements, unresponsive to external stimuli. No need for restrain.

Adapted from Schäffer et al. (2016).

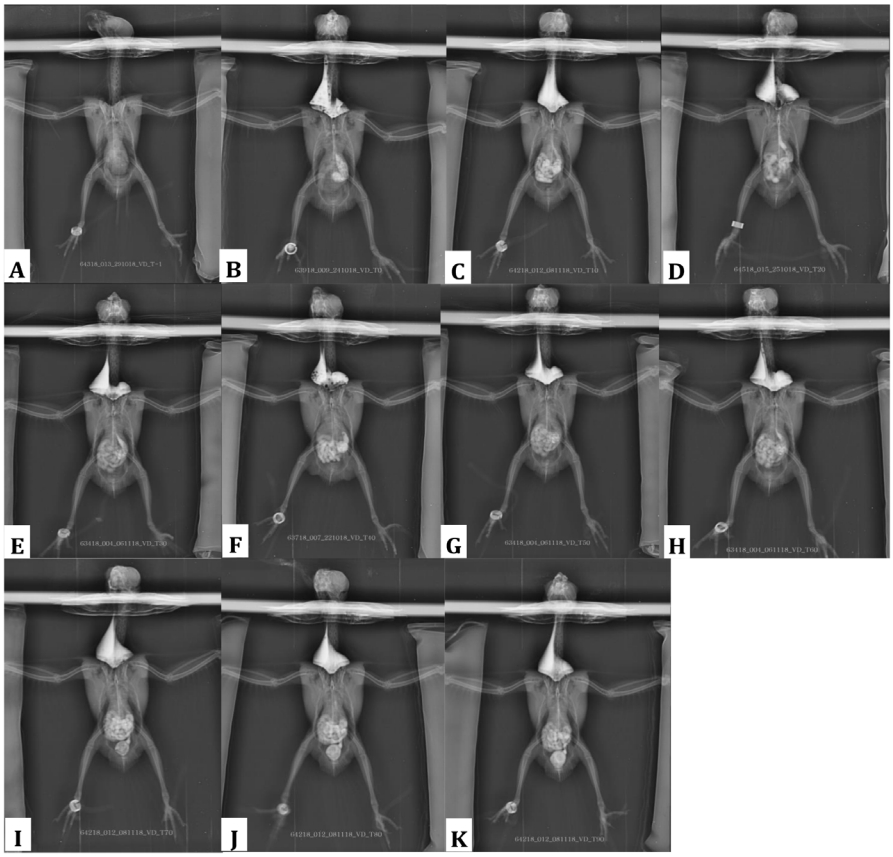

Figure 1 - Ventrodorsal radiographic images from 90 minutes of iohexol gastrointestinal transit study in adult cockatiels (Nymphicus hollandicus), at dose of 15 mL/kg, under sedation with intranasal midazolam (2 mg/kg). A: before contrast medium administration | B: 3 minutes after contrast medium administration) | C: 10 minutes after contrast medium administration | D: 20 minutes after contrast medium administration | E: 30 minutes after contrast medium administration | F: 40 minutes after contrast medium administration | G: 50 minutes after contrast medium administration | H: 60 minutes after contrast medium administration | I: 70 minutes after contrast medium administration | J: 80 minutes after contrast medium administration | K: 90 minutes after contrast medium administration.

presence of contrast medium at the cloaca, was 50 (30-90) minutes (Figures 1K and 2K). Six birds presented complete cloacal filling, whereas in the remaining birds, iohexol just highlighted the organ.

In this study, four birds were classified according to the visual sedation scale as scoring 2, three scored 3 and five scored 4.

DISCUSSION

All radiographic images obtained in this study enabled a satisfactory evaluation of the GI organs of the cockatiels. At a dose of 15 ml/kg, iohexol produced a suitable contrast for the evaluation of all segments, which is a lower dose than that used by ERNST et al. (1998) in mid-sized psittacine and MARTEL et al. (2018) in cockatiels, also other studies using barium sulphate (VINK-NOOTEBOOM et al., 2003; KUBIAK & FORBES, 2012; DOSS et al., 2017a, 2017b).

The radiographic technique used to obtain the images was appropriate for the species size and positioning device, was inexpensive and minimised the radiation exposure of people during study, in

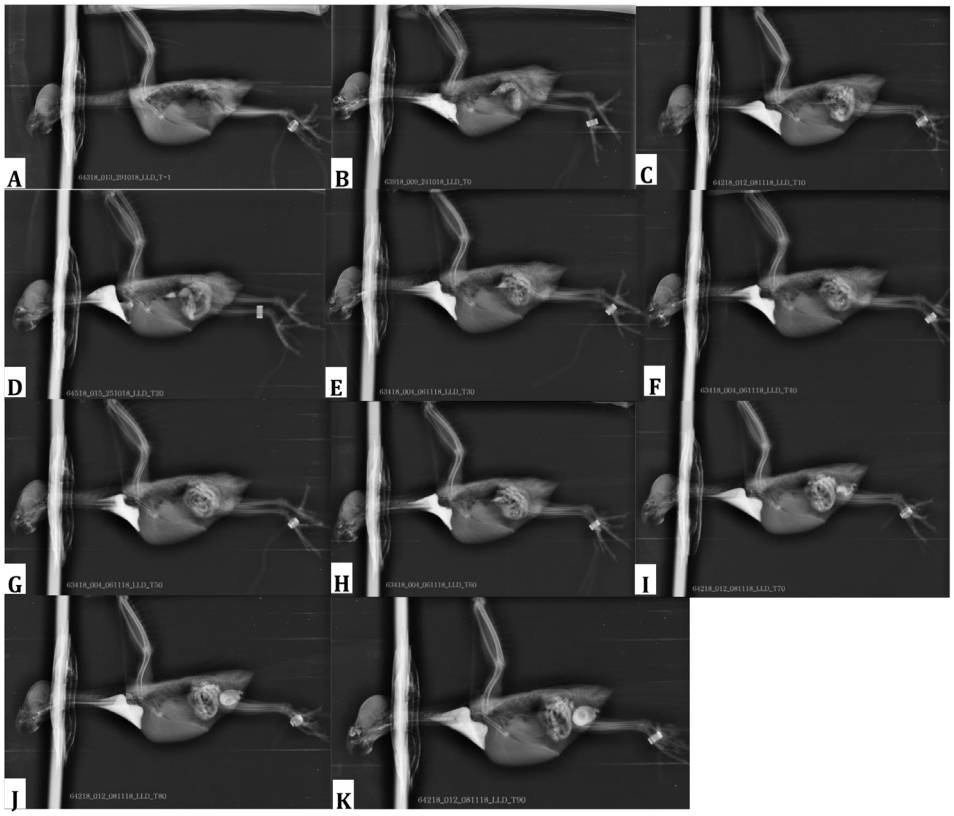

Figure 2 - Right lateral radiographic images from 90 minutes of iohexol gastrointestinal transit study in adult cockatiels (Nymphicus hollandicus), at dose of 15 mL/kg, under sedation with intranasal midazolam (2 mg/kg). A: before contrast medium administration | B: 3 minutes after contrast medium administration) | C: 10 minutes after contrast medium administration | D: 20 minutes after contrast medium administration | E: 30 minutes after contrast medium administration | F: 40 minutes after contrast medium administration | G: 50 minutes after contrast medium administration | H: 60 minutes after contrast medium administration | I: 70 minutes after contrast medium administration | J: 80 minutes after contrast medium administration | K: 90 minutes after contrast medium administration.

addition to maintaining a good position of the bird, as observed in previous studies (SILVERMAN & TELL, 2010b; NAGUIB, 2017).

Fasting is important before radiographic study since it increases the diagnostic value of the examination by improving image quality. However,in this study, as reported by ERNST et al. (1998) in a mid-sized psittacine, ventricular content visualized even after 4 hours of fasting did not significantly affect image quality.

According to the results obtained in this study, it is important to note that there is a significant individual variability of transit times, even in a group with similar characteristics and treatment. Factors including species, stress, drugs, age, diet, feeding behaviour, fasting before study, and contrast medium type and dilution have been reported to influence the passage of GI contrast medium in birds (ERNST et al., 1998; VINK-NOOTEBOOM et al., 2003; WAGNER & KIRBERGER, 2003; BEAUFRÈRE et al., 2010; KUBIAK & FORBES, 2012; DOSS et al., 2017a, 2017b; NAGUIB, 2017; MARTEL et al., 2018). Therefore, comparisons of the GI transit times in this population with those in other published reports in birds are challenging.

The GI times observed in this study were similar to those recorded in mid-sized psittacine birds without sedation using iohexol as the contrast

medium (ERNST et al., 1998). In the present study, the contrast medium reached the cloaca within 30 to 90 minutes, with a median of 50 minutes. These times are shorter than those observed in non-anaesthetised parrots and red-tailed hawks using barium sulphate as the contrast agent (VINK-NOOTEBOOM et al., 2003; KUBIAK & FORBES, 2012). In fact, despite the different species, a longer GI time is expected with barium sulphate than with iohexol (ERNST et al., 1998).

Emptying of the crop was not observed by the end of the study; it remained full of contrast medium over the time. In three birds, emptying of the proventriculus, ventriculus and duodenum was observed, even though the crop remained filled. Indeed, as noted by ERNST et al. (1998), in psittacines the crop acts a reservoir of contrast medium where iohexol remains throughout the imaging study, allowing continuous visualisation of the caudal GI organs, which creates a wide window for evaluating these organs in cockatiels.

A remarkable finding was that the oesophagus (cervical and thoracic) and isthmus presented a pattern of contrast progression different from those of the other GI segments. A fluctuation was observed between filling and emptying during the radiographic study (Figure 1B; 1K and figure 2B; 2K).

Considering that regurgitation of the contrast medium occurred in all birds at least once during the radiographic study, this could explain the pattern observed within the cervical oesophagus, even when elevating the bird's head during the examination.

Conversely, the thoracic oesophagus and isthmus can be considered 'transition areas'. The presence of contrast medium within these segments depends on ingluvial and proventricular emptying, respectively. As recorded in fluoroscopic studies in mid-sized psittacines (VINK-NOOTEBOOM et al., 2003; BEAUFRÈRE et al., 2010), there is a frequent passage of contrast medium from the crop through the thoracic oesophagus to the proventriculus, and also from the proventriculus through the isthmus to the ventriculus, by antegrade peristaltic waves. This explains the pattern observed within these organs in this present study.

Despite regurgitation being associated with the stress triggered by manual restraint during radiographic positioning (ERNST et al., 1998), it is important to note that the cockatiels presented moderate to intense muscular relaxation after intranasal administration of midazolam at a dose of 2 mg/kg. A previous study (DOSS et al., 2018) demonstrated the effects of intranasal midazolam at a dose of 3 mg/kg in cockatiels and concluded that it is an effective and safe option for sedating the species for procedures such as diagnostic imaging. However, the muscle relaxant effect (SCHÄFFER et al., 2016; SCHÄFFER et al., 2017), could explain the regurgitation of iohexol, which is a very liquid contrast medium (NAGUIB, 2017).

Another study reported dysmotility and longer GI transit time in cockatiels under sedation with intramuscular midazolam at dose of 6 mg/kg (MARTEL et al., 2018), which is a dose three times greater than used in the present study. The authors observed an overall transit time of 60 minutes for the control group in comparison with 90 minutes for the midazolam group. In the present study, most of the cockatiels (8/12) presented contrast medium within the cloaca up to 60 minutes.

It should be noted that intranasal midazolam has a wide margin of safety in birds and its effects, such as anxiolytic, muscle relaxant and sedative are proved to be dose-dependent. Thus, it is expected that higher doses cause greater effects (HEATLEY et al., 2015; SCHÄFFER et al., 2017; SCHÄFFER et al., 2016). However, there is no study comparing different doses of midazolam and their effects on GI function in birds.

Intranasal midazolam, as reported by DOSS et al. (2018), seems to be an appropriate sedation protocol for radiographic study in cockatiels, due to the ease of administration, its rapid sedative effect and recovery, its safety and the lack of any adverse effects in any bird. In this study, all birds in the VD position presented generalized muscle tremors, which was also observed by SCHÄFFER et al. (2016) in macaws, but this effect disappeared after recovery.

In this study, the optimal visualisation of GI organs depended on the radiographic positioning, as reported by other published studies in different species (WAGNER & KIRBERGER, 2003; SILVA et al., 2012; DOSS et al., 2017 [a b]). For example, this study demonstrated that in cockatiels the thoracic oesophagus is better evaluated in RL due to the superimposition of the sternum in VD. The proventriculus, isthmus and ventriculus are superimposed in VD, thus RL should be a better projection to evaluate these organs separately. However, when contrast medium reaches the small intestinal segments, they are superimposed on the ventriculus. In addition, visualisation of contrast medium within the cloaca can be difficult in RL due to superimposition of the legs.

After the study, no GI disorders or other adverse effects were observed in any bird. Iohexol proved to be a suitable and secure contrast medium for use in cockatiels at a dose of 15 ml/kg. As observed by ERNST et al. (1998) in psittacine birds, all cockatiels presented clear and watery stools at least 12 hours after radiographic study, due to GI emptying.

CONCLUSION

In healthy cockatiels under sedation with midazolam at a dose of 2 mg/kg, radiographic images of iohexol GI transit times should start to be obtained 3 minutes after contrast medium administration by crop gavage and continue for at least 60 minutes to observe cloacal filling.

Iohexol at a dose of 15ml/kg is a suitable contrast medium for use in cockatiels because no adverse effects were observed and adequate contrast was provided to evaluate all GI organs.

During all radiographic study, even with several positioning required, midazolam at a lower dose (2 mg/kg), proved to be an apropriete sedation protocol due to muscle relaxant and sedative effects, with rapid and safe recovery in cockatiels.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was reviewed and approved by the Ethics Committee on Animal Experimentation of the Universidade Federal da Fronteira Sul (CEUA/UFFS), protocol number 23205.000639/2018-21.

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

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