Journal of Veterinary Science and Public Health

J. Vet. Sc. Public Health, v. 11, n. 2, p. 066-082, 2024

EXPOSURE TO ULTRAVIOLET B RADIATION OR ORAL SUPPLEMENTATION WITH VITAMIN D AND CALCIUM IN LEOPARD GECKOS *EUBLEPHARIS MACULARIUS*: BENEFICIAL OR NOT?

(EXPOSIÇÃO À RADIAÇÃO ULTRAVIOLETA B OU SUPLEMENTAÇÃO ORAL COM VITAMINA D E CÁLCIO EM LAGARTIXAS-LEOPARDO *EUBLEPHARIS MACULARIUS*: BENÉFICO OU NÃO?)

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DOI 10.4025/revcivet.v11i2.64006

RESUMO

A vitamina D é responsável pela regulação de várias funções, como a homeostase do cálcio. Vários estudos têm pretendido compreender as necessidades de vitamina D de cada espécie, e a sua aquisição na natureza. O presente estudo teve como objetivo abordar a influência da suplementação com vitamina D e cálcio, ou exposição à radiação ultravioleta (UV) sobre os níveis de cálcio ionizado em Eublepharis macularius (lagartixa-leopardo). Amostras de sangue foram obtidas na veia jugular de 15 lagartixas-leopardo mantidas em condições ambientais distintas: animais suplementados, animais expostos à radiação UV e animais não suplementados ou expostos à radiação (controle). As amostras foram obtidas em dois check-ups, com diferença de 5 semanas, e os níveis de cálcio ionizado (iCa++) foram determinados. A concentração de iCa++ não foi diferente entre os check-ups ou entre os grupos (p>0,05). Os resultados sugerem que a lagartixa-leopardo pode manter a concentração de iCa++, sem suplementação, por pelo menos 42 dias. Além disso, parece que os animais não se beneficiaram da suplementação durante esse período.

Palavras-chaves: *Eublepharis macularius,* cálcio ionizado; radiação; colecalciferol.

ABSTRACT

Vitamin D is responsible for the regulation of several functions, like calcium homeostasis. Various studies have intended to understand the vitamin D needs of each species, and its acquisition in the wild. The present trial intended to address the influence of supplementation with vitamin D and calcium, or exposure to ultraviolet (UV) radiation on the levels of ionized calcium in *Eublepharis macularius* (Leopard gecko). Samples of blood were obtained in the jugular vein of 15 Leopard geckos maintained under distinct environmental conditions: animals supplemented, animals exposed to UV radiation and animals non-supplemented or exposed to radiation (control). Samples were obtained at two check-ups, with a difference of 5 weeks, and the levels of ionized calcium (iCa^{++}) were determined. The concentration of iCa^{++} was not different between check-ups or among groups ($p>0.05$). The results suggest that Leopard gecko can maintain the concentration of iCa^{++}, without supplementation, for at least 42 days. Moreover, it seems that the animals did not benefit from supplementation during this period.

Keywords: *Eublepharis macularius,* ionized calcium; radiation; vitamin D.

INTRODUCTION

The species *Eublepharis macularius* (Leopard gecko) was first described in 1854 by Edward Blyth and is currently one of the most popular lizard species kept as a pet, due to its docile temperament, ease of maintenance and breeding in captivity, its longevity and reduced size, and due to its beauty and diversity of colors and patterns (DE VOSJOLI et al., 2017). Scientifically, it is a species of great interest due to its regeneration capacities (PEACOCK et al., 2015; NAKASHIMA, 2016; MCDONALD et al., 2018) and its thermo-dependent sexual determination and behavior (HUANG et al., 2012; HUANG et al., 2014).

Vitamin D regulates several physiologic functions in vertebrates, like calcium homeostasis, playing an active role in bone remodeling, intestinal calcium absorption, and renal calcium reabsorption. This vitamin can be obtained by eating aliments naturally containing it, mushrooms or plants exposed to ultraviolet (UV) radiation, foods fortified with vitamin D or through food supplements (HOSSEIN-NEZHAD and HOLICK, 2013). Alternatively, vitamin D3 (or cholecalciferol) can be produced in the skin, through exposition to UV B radiation followed by a thermo-dependent process. After its production in the skin, vitamin D3 is moved to the liver where it suffers a hydroxylation on carbon 25, by the enzyme 25-hydroxylase. 25-hydroxycholecalciferol, 25-hydroxyvitamin D3 or calcidiol (25 (OH) D3) is the most abundant vitamin D metabolite in the blood and is not biologically active, however, it plays a very important role as a reserve of this vitamin (DAHLBACK et al., 1988; CLINE, 2012; HOLICK, 2014; JONES et al., 2018; OONINCX et al., 2020). To become biologically active, this form of vitamin D must experiences a second hydroxylation, mediated by the enzyme 1α-hydroxylase mainly in the kidneys, resulting in the formation of 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), 1,25-dihydroxycholecalciferol or calcitriol, that is the active form of vitamin D3 (ZEHNDER et al., 1999; ZEHNDER et al., 2001; ZEHDNER et al., 2002; CLINE, 2012; HOLICK, 2014; LARNER et al., 2018).

Vitamin D intoxication and deficiency can lead to several adverse effects. Vitamin D intoxication can be difficult to diagnose, due to the slow appearance of clinical signs and, consequently, the prognosis can be very poor (CLINE, 2012). This intoxication may cause soft tissue mineralization (gastrointestinal tract, muscles, kidneys, lungs, heart, and great vessels) that can be seen in radiograph or ultrasound exams, and in the long term can cause bone malformations. These mineralizations can cause pain and organic dysfunction or failure (WALLACH, 1996; RAITI and GARNER, 2006; O'MALLEY, 2008; CLINE, 2012; PENNING, 2012; WATSON, 2014; BOSKEY, 2018; BOYER and SCOTT, 2019). Metabolic bone disease (MBD) caused by secondary nutritional hyperparathyroidism is the main consequence of hypovitaminosis D in reptiles (WATSON, 2014). When vitamin D deficiency is continued, the bone tissue destroyed during resorption is not recuperated, causing a decrease in bone density (osteopenia) and weakening bones (LOCK, 2017). Thus, the most common clinical signs include bone malformations, pathological fractures, swelling and stiffness of the hind limbs, difficulty in lifting the body or tail, rubber jaw, tremors, paresis or paralysis of the limbs, and lethargy (O’MALLEY, 2008; PENNING, 2012; LOCK, 2017).

Due to the enormous diversity of reptile species and the environmental adaptations, several studies have been performed to understand the individual needs and adaptations of each species regarding vitamin D, its importance and acquisition in the wild (VERGNEAU-Grosset et al., 2020). In general, herbivorous and insectivorous reptiles need UV radiation to obtain vitamin D, as the concentration of this vitamin in their food is very low or nonexistent (SELLERI et al., 2012).

A study performed on leopard geckos showed that these animals, when exposed to short periods of UV radiation (2 h), have the capacity to significantly increase the concentration of 25-hydroxyvitamin D3, suggesting that crepuscular and nocturnal species may have the capacity to produce vitamin D3 when exposed to UV radiation, even if during short periods (GOULD et al., 2018). Indeed, it seems that several reptile species with lower sunlight opportunity developed an adaptation mechanism that allows them to synthesize vitamin D3 more efficiently (CARMAN et al., 2000; FERGUSON et al., 2005; BAINES et al., 2006;

RUTLAND et al., 2019). A study conducted in ball pythons found no association between exposure to UV-b radiation and plasma 25-hydroxyvitamin D3 and ionized calcium concentrations (HEDLEY et al., 2013). Inversely, a study developed in bats, suggested that the species that roosts in darker places developed a higher sensitivity to UV radiation and, consequently, a higher 7-dehydrocholesterol conversion rate (SOUTHWORTH et al., 2013). So, the importance of UV radiation on nocturnal/crepuscular species continues to be questioned.

To contribute to the field, this study intended to address the influence of exposure to UVB radiation or oral supplementation with vitamin D and calcium on plasma levels of ionized calcium in leopard geckos (*Eublepharis macularius*).

MATERIALS AND METHOD

This study was previously approved by the Ethics and Animal Welfare Commission (CEBEA) of the Faculty of Veterinary Medicine, of the Lusophone University of Humanities and Technologies.

Animals: Blood samples were collected in check-up appointments, in 15 leopard geckos from different owners and submitted to different environmental conditions: supplemented animals (n=5), animals subjected to UV radiation (n=5) and animals not supplemented or subjected to radiation (control) (n=5). The leopard geckos were housed individually in plastic boxes, measuring 56cm x 39cm x 28cm. Each box had two shelters on opposite sides, a container with water, a container for food and paper towels to facilitate cleaning. The boxes were put over an electric heating mat that occupied one-third of the base, maintaining the temperature, in the hot zone, at 30ºC (Figure 1A). All geckos were fed with mealworms, crickets and/or cockroaches, two to three times a week. The food of the group that received supplements was pulverized with calcium and vitamin D3 supplements (Exo Terra Calcium + D3 Powder Supplement, Exo Terra, Rolf C. Hagen, Inc., Montreal, Canada). The geckos exposed to radiation had a 13W Mega Sun UVB 10.0 compact fluorescent lamp from Reptiles Planet (Paris, France) 30 cm above its base, connected to a timer programmed to switch on between 10 am and 6 pm. The remaining geckos did not receive supplements nor were exposed to radiation, being used as a control group. In the week prior to the initial check-up, animals did not receive supplements or were exposed to radiation.

Samples collection: The animals were weighed in both check-up visits, using a scale. Blood samples were obtained from all animals at the initial and final check-ups, five weeks after the initial check-up. All samples were collected by puncture of the jugular vein, using BD Micro-Fine ™ + 0.33 mm (29G) x 12.7 mm insulin syringes (BD Medical - Diabetes Care Becton Dickinson, Le Pont-de-Claix, France), to a heparin tube (BD Vacutainer™ Plastic Blood Collection Tubes, VWR, Avantor, USA), between 10 am and 12 pm (Figure 1B). All samples were analyzed up to one hour after collection using the portable blood gas, electrolyte, chemistry and hematology analyzer VetScan® i-STAT®1 (Abaxis, Inc., Union City, California, United States of America), using the VetScan® i-STAT®1 CG8 + that analyzes the following parameters: pH, partial pressure of carbon dioxide (PCO2), partial pressure of oxygen (PO2), excess of base (BE), bicarbonate (HCO3-), total carbon dioxide (TCO2), oxygen saturation (sO2), sodium ion (Na+), potassium ion (K+), ionized calcium (iCa++), glucose (Glu), hematocrit (Hct) and hemoglobin (Hgb).

Statistical analysis: The data collected were statistically analyzed with the SPSS® version 26 (IBM, Armonk, New York, USA), using the Wilcoxon nonparametric test. Values are presented as mean ± standard deviation (S.D.). The differences were considered statistically significant for values of $p<0.05$.

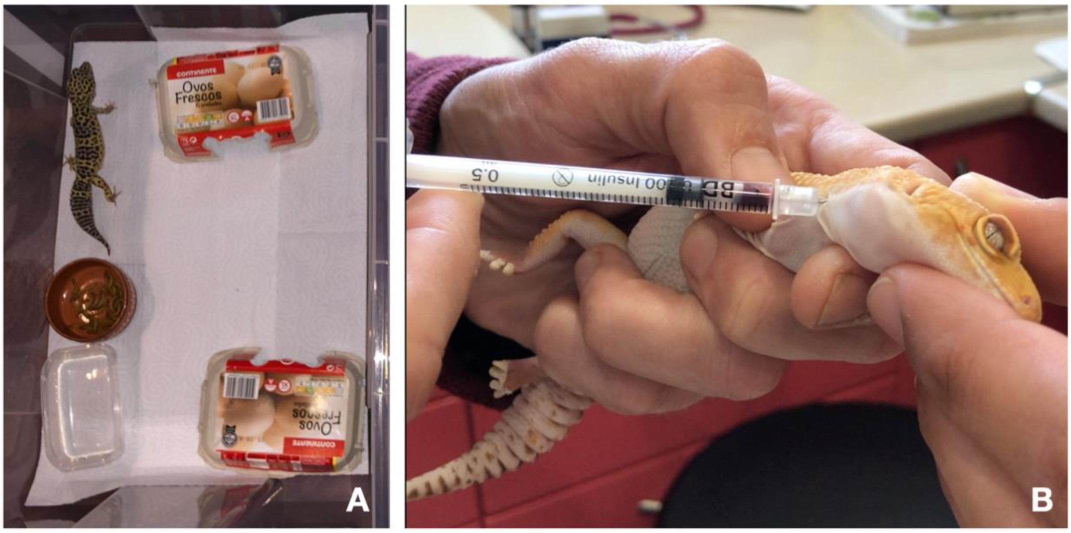

Figure 1. A) Example of a plastic box where the geckos were housed individually. B) Blood sample collection through the venopuncion of the right jugular vein.

RESULTS

Animals: Table 1 shows the distribution of male and female leopard geckos at the beginning of the study. Two geckos were excluded from the study before the second check-up: a female from the group exposed to radiation and a male from the control group. Three weeks after the initial check-up, the female from the group exposed to radiation was excluded from the study because she had a decrease in body mass of approximately 25%, dysecdysis and the skin in poor condition. Five days before the final check-up, a male from the control group was excluded for presenting clinical signs of MBD. Data from these animals were not included in the study.

Table 1. Animals in each group and their age (mean ± S.D.) at the beginning of the study.

Group	Supplemented (n=5)	Radiation (n=5)	Control (n=5)
Male (n)	3	3	2
Female (n)	2	2	3
Age (months)	38.0 ± 27.9	28.8 ± 18.2	27.4 ± 24.4

The mean initial and final body mass of each group can be seen in Table 2. The animals from the control group were those with the lowest initial and final body mass (35.25 ± 9.06 g and 40.75 ± 9.29 g). The owners of the geckos exposed to radiation reported that these animals had a decreased appetite since the first week of exposure. The body mass of these animals decreased slightly between the initial and final check-up visits (62.75± 9.36 g and 62.00 ± 16.43 g, respectively). However, the mean body mass did not vary significantly within each group between the beginning and the end of the study (supplemented group: p=0.854; group exposed to radiation: p=0.854; control group p=0.141). Statistically significant differences in body mass were not observed among groups (p>0.05).

Table 2. Body mass (g) at the beginning and end of the study (mean ± S.D.).

Group	Supplemented (n=5)	Radiation (n=4)	Control (n=4)
Initial body mass	58.60 ± 6.10	62.75 ± 9.36	35.25 ± 9.06
Final body mass	59.00 ± 6.93	62.00 ± 16.43	40.75 ± 9.29

Ionized Calcium: Table 3 shows the mean iCa++ blood concentration value for each group at the initial and final check-ups. An increase in iCa++ blood concentration was observed in all groups between the initial and final check-ups. However, this variation was not statistically significant (supplemented group: p=0.500; group exposed to radiation: p=0.461; control group: p=0.144). There were no statistically significant differences in blood iCa concentration among groups (p>0.05).

Table 3. Ionized Calcium blood concentration (iCa) (mmol/L) at the beginning and end of the study (mean ± S.D.).

Group	Supplemented (n=5)	Radiation (n=4)	Control (n=4)
Initial iCa^{++}	1.41 ± 0.14	1.31 ± 0.07	1.27 ± 0.08
Final iCa^{++}	1.46 ± 0.12	1.40 ± 0.11	1.42 ± 0.17

Table 4 shows the mean values of the remaining parameters analyzed: Na +, K +, Glu, Hct, Hgb, pH, PCO2, PO2, BE, HCO3-, TCO2 and sO2.

Blood Na+ concentration increased in all groups between the beginning and the end of the study, however the differences were not statistically significant (*p*>0.05). Conversely, the blood K+ concentration decreased between the beginning and the end of the study, however the differences did not reach the level of statistical significance (*p*>0.05). The blood Glu concentration did not vary significantly between the beginning and the end of the study (*p*>0.05). Hct values increased in the three groups between the beginning and the end of the study, however the differences were not statistically significant (*p*>0.05). Hgb blood concentrations increased in all groups between the beginning and the end of the study, but not significantly (*p*>0.05). In the remaining parameters (pH, PCO2, PO2, BE, HCO3-, TCO2 and sO2) there were no significant variations between the initial and final values within each group (*p*>0.05). None of the evaluated parameters showed statistically significant differences among groups (*p*>0.05).

Table 4. Initial and final values of Na+ (mmol/L), K+ (mmol/L), glucose (mg/dL), hematocrit (%PCV), hemoglobin (g/dl), pH (a 37ºC), pCO2 (mmHg), pO2 (mmHg), BE (mmol/L), HCO3 (mmol/L), TCO2 (mmol/L) e sO2 (%) (mean ± S.D.).

Group	Supplemented (n=5)	Radiation (n=4)	Control (n=4)
Initial Na^+ (mmol/L)	126.80 ± 5.40	126.00 ± 2.94	120.00 ± 6.16
Final Na^+ (mmol/L)	135.00 ± 10.05	132.75 ± 14.45	127.00 ± 12.93
Initial K^+ (mmol/L)	4.26 ± 1.16	3.60 ± 0.50	5.32 ± 1.02
Final K^+ (mmol/L)	4.20 ± 0.51	3.48 ± 0.31	3.78 ± 0.22
Initial Glucose (mg/dl)	130.20 ± 42.80	103.50 ± 19.47	114.50 ± 19.12
Final Glucose (mg/dl)	128.40 ± 20.76	117.25 ± 12.26	114.50 ± 13.80
Initial Hematocrit (%PCV)	26.20 ± 2.95	24.75 ± 2.22	22.25 ± 4.50
Final Hematocrit (%PCV)	35.00 ± 10.22	25.25 ± 11.30	24.50 ± 10.22
Initial Hemoglobin (g/dl)	8.90 ± 1.02	8.50 ± 0.89	7.55 ± 1.54
Final Hemoglobin (g/dl)	11.92 ± 3.49	9.73 ± 3.76	8.35 ± 3.49
Initial ph (37ºc)	7.40 ± 0.19	7.51 ± 0.10	7.46 ± 0.15
Final ph (37ºc)	7.40 ± 0.20	7.58 ± 0.17	7.31 ± 0.17
Initial pco2 (mmhg)	20.62 ± 3.27	20.50 ± 2.07	19.93 ± 4.09
Final pco2 (mmhg)	21.26 ± 4.12	20.13 ± 4.72	17.13 ± 6.12
Initial po2 (mmhg)	109.60 ± 62.52	120.75 ± 48.31	145.25 ± 67.49
Final po2 (mmhg)	93.20 ± 28.08	116.50 ± 44.47	115.00 ± 47.07
Initial BE (mmol/L)	-11.20 ± 8.64	-6.25 ± 5.06	-9.50 ± 5.07
Final BE (mmol/L)	-11.40 ± 7.09	-3.50 ± 5.80	-16.75 ± 7.04
Initial HCO3 (mmol/L)	13.66 ± 5.57	16.60 ± 3.51	14.25 ± 2.52
Final HCO3 (mmol/L)	13.46 ± 3.89	18.65 ± 2.90	9.25 ± 4.59
Initial TCO2 (mmol/L)	14.40 ± 5.46	17.25 ± 3.30	14.75 ± 2.63
Final TCO2 (mmol/L)	14.20 ± 3.70	19.25 ± 2.87	10.00 ± 4.16
Initial so2 (%)	97.20 ± 1.79	98.50 ± 1.73	99.25 ± 0.96
Final so2 (%)	96.80 ± 1.30	98.75 ± 1.26	97.00 ± 2.58

DISCUSSION

Vitamin D is responsible for regulating the blood concentration of calcium and phosphorus (FLEET, 2018). Most vertebrates produce vitamin D3 in the skin through exposure to UV radiation (HOLICK et al., 2014). The importance of UV radiation in the production of vitamin D3 in nocturnal or crepuscular species continues to be examined. Several studies show that some of these species can produce vitamin D3 in the skin and that diurnal species with less chance of exposure to sunlight have adapted and are able to synthesize vitamin D3 more effectively (CARMAN et al., 2000; BAINES et al., 2006; FERGUSON et al., 2015; BOS, et al., 2018; GOULD et al., 2018; RUTLAND et al., 2019). Studies performed on leopard geckos concluded that they can synthesize vitamin D3 endogenously. Geckos exposed to short periods of UV radiation significantly increased calcidiol plasmatic levels when compared to those without exposure or supplements (GOULD et al., 2018). Another study concluded that leopard geckos exposed to UV radiation increased calcidiol concentration to values 3.2 times higher than animals receiving supplements (WANGEN et al., 2013). However, the physiological importance of higher calcidiol levels in this species remains unknown.

In a study conducted by WANGEN et al. to assess the influence of UV radiation on the blood concentration of calcidiol in leopard geckos, it was shown that these animals, when exposed to 12 hours of daily UV radiation, increased the concentration of calcidiol to values significantly higher than those receiving only oral supplementation (GOULD et al., 2018; BOYER and SCOTT, 2019). GOULD et al. (2018) also performed a study to address the influence of UV radiation on the blood concentration of calcidiol in leopard geckos and concluded that this species has the ability to endogenously synthesize vitamin D3 when exposed to short periods of UV radiation (2 hours) and increase their values significantly when compared to those animals neither exposed to radiation nor supplemented orally with vitamin D or calcium. Our study lasted for thirty days and, with the exception of the male and female that were excluded, no other animals exhibited adverse effects (GOULD et al., 2018). In the present study, exposure to UV radiation or oral supplementation with vitamin D and calcium did not significantly increase the concentration of iCa^{2+}, when compared to the animals without any supplementation or access to UV radiation.

A female leopard gecko from the group exposed to UV radiation was excluded three weeks after the beginning of the study for presenting a sharp decrease (about 25%) in body mass, dysecdysis and poor skin conditions. In addition to these changes, owners noticed a decrease in the appetite of the animals in this group and, although the decrease was not statistically significant, the mean body mass of the group exposed to UV radiation decreased during the study. This effect was not seen in any of the animals from the supplemented group or in the control group, suggesting that this decrease in appetite may be related to UV radiation exposure. Although it is rare, there are several reported cases of vitamin D toxicity in reptiles. Clinical signs are not specific and include depression, anorexia, and polyuria/polydipsia (RAITI and GARNER, 2006; BOYER and SCOTT, 2019). However, there are no reported cases of vitamin D poisoning in animals exposed to UV radiation due to the defense mechanisms developed by the body to prevent excess cutaneous production of cholecalciferol (HOLICK et al., 1981; MACLAUGHLIN et al., 1982; WEBB et al., 1989). Additionally to decreased appetite, geckos exposed to radiation showed no other signs associated with vitamin D intoxication. A study carried out on budgerigars (*Melopsittacus undulatus*) reported that the exposure of these animals to UV radiation causes several adverse effects, and the decrease in the animals' body mass was one of the effects associated with exposure to low intensity UV radiation (LUPU et al., 2013). The exposure of the skin to UV radiation stimulates the production of the α-Melanocyte stimulating hormone (α-MSH) which is responsible for activating melanin production and suppressing appetite (RODRIGUES et al., 2019; BALDINI and PHELAN, 2019). After UV radiation exposure, an increase in α-MSH levels is expected, with a possible decrease in appetite and consequent decrease in body mass. UV radiation has other possible adverse effects, like skin burns that cause discomfort and pain (LOPES and MCMAHON, 2015). In an unpublished study on the influence of UV radiation on leopard geckos, Mitchell noted that animals exposed to radiation increased the frequency of skin shedding and developed skin lesions consistent with mild sunburn (WATSON, 2014). It is possible that the poor general condition observed on the skin of the female leopard gecko excluded from the study was due to a skin burn caused by radiation. This may have been due either to excessive exposure time or to exposure to radiation with high intensity. However, this animal, unlike the geckos of the study carried out by Mitchell, did not increase the frequency of skin shedding and, in fact, was not doing it correctly. With the exception of this animal, none of the other animals exposed to UV radiation showed clinical skin signs.

The other gecko excluded from this study was a young male (about five months old) from the control group that started showing clinical signs of MBD 30 days after the beginning of the study. MBD is a term used to describe a set of diseases that affect the integrity and function of bones. The most common MBD in reptiles is nutritional secondary hyperparathyroidism, which can have several causes, with a common cause being vitamin D or calcium deficiency (CARMEL and JOHNSON, 2017; LOCK, 2017). The gecko excluded from the study did not have access to UV radiation and did not receive supplements, which could result in a deficiency of vitamin D and, consequently, of calcium. However, the development of clinical signs depends on a prolonged deficiency (BOYER and SCOTT, 2019), and the remaining animals deprived of UV radiation or supplements did not develop symptoms. The development of clinical signs of MBD in this gecko may be related to its age, once young animals and animals in active growth have a greater need for calcium and, for this reason, are more predisposed to develop MBD (CARMEL and JOHNSON, 2017; BOYER and SCOTT, 2019). The remaining animals in the control group were over one year old, possibly having a lower calcium demand.

The main veins used to collect blood samples from lizards are the ventral or lateral coccygeal vein, the ventral abdominal vein, and the external jugular vein (DIVERS, 2019). The ventral or lateral coccygeal vein should be avoided in species that are able to perform caudal autotomy, like Leopard geckos (DI GIUSEPPE et al., 2017; KNOTKOVA et al., 2019). Blood collection through the ventral abdominal vein has possible complications, such as perforation of the gastrointestinal tract or bladder and hemorrhages, due to the difficulty of pressing the vein to ensure a correct hemostasis after the collection (DIVERS, 2019). Cardiac venopunction is not safe in lizards due to the difficulty of stabilizing the heart (DI GIUSEPPE et al., 2017; DIVERS, 2019; KNOTKOVA et al., 2019). In 2017, DI GIUSEPPE et al. described a technique for collecting blood through the external jugular vein of small lizards, in which the animal must be restrained manually with the neck and head extended and with a slight rotation to the contralateral side to the external jugular vein where the sample will be collected, and the ipsilateral anterior limb to the jugular vein must be pulled caudally (DI GIUSEPPE et al., 2017). Thus, the method chosen was the collection through the external jugular vein, since this technique is safe and fast, and the animal is easily restrained. The blood volume of reptiles varies between 5 and 8% of the animal's body mass. The sample collected should not exceed 0.4 to 0.8 mL per 100 g of body weight in healthy reptiles (DIVERS, 2019).

There is no consensus on the influence of calcidiol on intestinal calcium absorption. Some studies have concluded that an increase in blood concentration of 25(OH)D results in an increase in intestinal calcium absorption (HEANEY et al., 1997; HEANEY et al., 2003; ALOIA et al., 2013), while other authors found no relationship between the two factors (LEWIS et al., 2013; HANSEN et al., 2015). NEED et al., (2008) showed that intestinal calcium absorption only accompanies the increase in calcidiol concentration while this concentration is insufficient to maintain calcitriol production at the renal level, suggesting that the increase in calcidiol concentration to values above necessary for the proper production of calcitriol may not influence intestinal calcium absorption. Thus, assuming that animals exposed to radiation significantly increased the blood concentration of calcidiol when compared to groups not exposed, as suggested by studies conducted by WANGEN et al. (2013) and GOULD et al. (2018), it is possible that there was not a significant difference in the blood concentration of iCa^{2+} between the groups because the calcidiol reserves of the unexposed geckos were sufficient to maintain adequate calcitriol production, ensuring a sufficient absorption of calcium. In the study conducted by GOULD et al. (2018), geckos were exposed to two hours of daily radiation that resulted in a significant increase in the blood concentration of calcidiol. In the present study the exposure was eight hours daily, which may have led to the photodegradation of the vitamin D produced. UV radiation can degrade up to 95% of the vitamin D produced in a period of three hours (WEBB et al., 1989). However, WANGEN et al. (2013) exposed leopard geckos to 12 hours of daily radiation and also observed a significant increase in the concentration of calcidiol suggesting that, even with photodegradation, skin production would be sufficient to significantly change the values.

The animals that received oral supplementation did not significantly increase the iCa^{2+} concentration. This may have happened because oral supplementation was not adequate, or, again, because the reserves of calcidiol were already sufficient to maintain the required production of calcitriol. When the live food is pulverized with the powdered supplement (a method used in the study and recommended by the brand itself), if it is not immediately eaten, it may lose part or all of the supplement (LI et al., 2009).

The animals in the control group did not receive vitamin D and calcium supplementation, nor were they exposed to UV radiation. Thus, they had no access to sources of vitamin D and did not have the opportunity to produce it. However, they did not show variation in the iCa^{2+} values during the study period, which means, they were able to maintain these values despite having no way of obtaining vitamin D. Once again it is possible that the vitamin D reserves of these animals were sufficient to maintain adequate production of calcitriol and, consequently, maintain the levels of iCa^{2+} necessary for the physiological functions of the leopard geckos. Bearded Dragons are able to maintain blood concentration of both calcidiol and calcitriol for a period of up to 83 days after the end of UV radiation exposure (OONINCX et al., 2013). This study lasted 35 days and in the week before the first check-up, no animals received supplements or were exposed to UV radiation, suggesting that the leopard gecko is capable to ensure the blood concentration of iCa^{2+} and possibly the concentration of the vitamin D metabolites, for at least 42 days. However, the amount of calcium present in the control group's diet would hardly be sufficient to maintain adequate calcium levels (BERNARD et al., 1997; LI et al., 2009) without resorting to bone resorption and causing a possible MBD. The short duration of the study may have conditioned the appearance of clinical signs of MBD in this group.

CONCLUSION

The results obtained in this study suggest that leopard geckos *Eublepharis macularius* have the ability to maintain iCa++ levels, without any type of supplementation, for at least 42 days. Geckos exposed to UV radiation or with oral vitamin D and calcium supplementation, despite a likely increase in the blood concentration of calcidiol, did not increase the blood concentration of iCa ++. New studies should be carried out in the future to assess the importance and influence of UV radiation and oral supplementation in maintaining the concentration of iCa ++ in the long term, so that it is possible to continuously improve the environmental conditions of these animals, which are increasingly common in captivity.

ACKNOWLEDGMENTS

This work was supported by National Funds by FCT - Portuguese Foundation for Science and Technology, under the project UIDB/04033/2020 (https://doi.org/10.54499/UIDB/04033/2020).

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