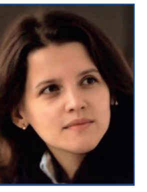
The lifestyle of the leopard gecko and the importance of ultraviolet radiation, vitamin D and calcium



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Abstract

The gecko leopard (*Eublepharis macularius*) was first described in 1854 by British herpetologist Edward Blyth. It is one of the most popular pet lizard species, due to its docile temperament, ease of maintenance and reproduction in captivity, its high longevity and small size, and its beauty and diversity of colours and patterns. Ultraviolet (UV) radiation is an important behavioural regulator. Vitamin D also regulates many fundamental physiological functions in vertebrates, mainly calcium homeostasis. Because of the great diversity of reptile species and the wide range of environmental adaptations, it is important to know the necessities and adaptations of each species regarding UV, vitamin D and calcium requirements. This study provides an overview of the leopard gecko's lifestyle, and the importance of ultraviolet radiation, vitamin D and calcium for this species.

Key words: *behaviour; Eublepharis macularius; habitat; life; supplementation*

Leopard gecko

Eublepharis macularius was first described in 1854 by Edward Blyth, a British herpetologist (Khan, 2016). The genus *Eublepharis*, which was first described in 1827 by the British zoologist John Edward Gray, results from the combination of the words "eu", meaning "good" or "true", and "blephar", meaning "eyelid", and the main characteristic of this genus is the presence of movable eyelids. The word "macularius" means "spotted" (Mirza et al., 2014; de Vosjoli et al., 2017). Commonly known as the leopard gecko, it is one of the most popular lizard species as a pet, mainly due to its docile temperament, ease of maintenance and reproduction in captivity, its high longevity and small size, and its beauty and di-

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versity of colours and patterns (de Vosjoli et al., 2017). There are several records of these animals living for more than 20 years in captivity, and the maximum age recorded in this species is 28 years and six months at the St. Louis Zoo (Shifter, 1988; Slavens and Slavens, 2001). From the scientific point of view, it is a species of great interest and frequently studied due to its regeneration ability (McLean et al., 2011; Delorme et al., 2012; Peacock et al., 2015; Nakashima, 2016; McDonald et al., 2018) and its thermo-dependent sexual determination and behaviour (Viets et al., 1993; Flores et al., 1994, 1995; Rhen et al., 2000; Putz et al., 2005; Pokorná et al., 2010; Huang et al., 2012, 2014).

Taxonomy

Based on the currently accepted taxonomic framework, *Eublepharis macularius* belongs to the Phylum *Chordata*, Sub-phylum *Vertebrata*, Class *Reptilia*, Order *Squamata*, Suborder *Sauria*, Family *Gekkonidae*, Subfamily *Eublepharinae*, Genus *Eublepharis* and Species *Eublepharis macularius* (Khan, 2016). They are born about 8 cm in length and in the adult stage length varies between 20 and 25 cm and weight between 45 and 60 g. Generally, males are larger than females and have 9 to 14 pre-cloacal pores arranged in an arc shape and two protuberances at the base of the tail. In the wild, colouration varies from straw yellow to pinker tones with black spots. However, due to their high popularity, and selections and crosses made by breeders, there are more than 160 mutations and combinations of different colours, patterns, and sizes (Kratochvíl and Frynta, 2002; Khan, 2016; de Vosjoli et al., 2017).

Habitat

The natural habitat is distributed across Pakistan, Iran, Afghanistan, India, and Nepal (Khan, 2016; Rawat et al., 2019). In urbanised areas, they colonise holes and cracks in man-made structures, such as walls, roads, bridges and spaces close to underground pipes that provide the necessary moisture and shelter (Khan, 2016). They also inhabit rocky rural areas with bushes and shelter in holes and cracks in the soil, rocks, and stones. They avoid deserts and prefer humid places. They are nocturnal animals, leaving the shelter at dusk to search for food alone, and returning before dawn (Khan, 2016). In its natural habitat, in Pakistan, temperatures vary between 22-24°C in March and 40-45°C in June and July, and the relative humidity varies between 30-40%. During the monsoon season, temperatures drop to 28-33°C and humidity rises to 70-80%. Between March and June, the humidity in the shelters remains between 40 and 56%, which is ideal for these animals (Khan, 2016). In colder areas, they hibernate from September until March, while in warmer areas hibernation may be delayed until November or may be absent (Khan, 2016).

Social organisation and reproduction

Gecko colonies, before they reach sexual maturity (pre-breeding colony), are made up of several young males and females. However, when they reach sexual maturity, they become aggressive and territorial, leading to the dispersion of males, leaving only the most dominant with several females (breeding colony). The reproductive season extends from March to July and, generally, females lay two eggs per clutch (Khan, 2016). The sex determination of this species is thermo-dependent. Thus, when incubation takes place at lower temperatures (26 to 30°C) and higher (34 to 35°C) exclusively or mostly females are born, while at intermediate temperatures (31 to 33°C) mostly males are born. The incubation period is inversely proportional to the incubation temperature, between 26°C and 32.5°C, varying between 36 and 72 days (Viets et al., 1993).

Food

Leopard geckos are mainly insectivores, feeding on beetles and grasshoppers, spiders, scorpions, and centipedes. As they age, they become opportunistic predators and attack smaller geckos, some snakes, newborn mice and bird nests (Khan, 2016). A case of cannibalism has been described in young animals (about 2–3 months old) kept in captivity (Bonke et al., 2011).

Threats in Nature

In its natural habitat, the leopard gecko is preyed on by various wild animals, such as foxes, jackals, mongooses, owls, kites, varanids and other lizards, and most snakes present in the same areas (Khan, 2016). In addition to these predators, and despite being one of the most popular pet reptiles in the world, in some areas of its natural habitat, local people consider this species to be a poisonous animal, believing it is related to the snakes *Naja naja karachiensis* and that its bite liquefies the victim's body, causing instantaneous death. For this reason, many of these native peoples kill geckos upon sight (Khan, 2016). There is currently no information available about the conservation status of this species in its natural habitat (IUCN Red List, 2020).

Captivity

According to Wright (2011, as cited by Boyer, 2013), the leopard gecko is the second most common reptile species in veterinary practice (13% of reptile cases). Most cases result from poor conditions in captivity. Some of the most common pathologies are nutritional pathologies (nutritional hyperparathyroidism, hypovitaminosis A), pathologies associated with deficiencies in skin shedding (dysecdysis), pathologies of the gastrointestinal tract (gastrointestinal obstructions and other pathologies in the gastrointestinal system, often associated with cryptosporidiosis), pathologies of the reproductive system, trauma (including tail autotomy), ocular pathologies and subcutaneous abscesses (Boyer, 2013; Miles, 2017). When kept in captivity, as terrestrial animals, the terrarium should be horizontal and as large as possible. However, the minimum acceptable volume of a terrarium to keep a specimen is about 38 litres, with the length of the terrarium at least twice the length of the animal and the width at least equal to the length of the animal (Bartlett, 1995; Wilkinson, 2015; deVosjoli, 2017). Various artificial or natural materials can be used as substrate. Artificial substrates, such as kitchen paper or newspaper sheets, are advantageous due to their low price and easy cleaning, however, they are less aesthetically appealing than natural substrates. If natural substrates are chosen, the risk of intestinal impaction must be considered and substrates that are not particulate or easily digestible must be chosen (Wilkinson, 2015; de Vosjoli et al., 2017). Leopard Geckos have an average ideal body temperature of 28.2±0.6°C, increasing significantly throughout the day (Angilletta et al., 1999). A thermal gradient must be maintained in the terrarium, to allow the animals behavioural thermoregulation, optimising metabolism and stimulating growth (Autumn, 1995). Thus, the animal must be provided with an area of the terrarium with a temperature between 29 and 33°C, and a colder zone at about 24°C. During the night the temperature can drop to 18° if a warm spot is maintained in the terrarium. This can be done, for example, by placing a heating mat under the terrarium that occupies 25 to 35%

of its area (de Vosjoli et al., 2017). It is also important to maintain an adequate relative humidity gradient to facilitate ecdysis or oviposition and to prevent chronic dehydration. The most frequently used method consists of placing a substrate that can maintain humidity (for example a substrate of Sphagnum moss) in a plastic box and making an opening in the lid so that the animals can get in and out (Rossi, 2019). Feeding must be based on insects or other live invertebrates, as these provide the animals with important environmental enrichment and can prolong the feeding period. However, most foods available on the market are nutritionally unbalanced if not supplemented in an appropriate way. For example, insects in the larval stage generally have a disproportionately higher amount of fat than the amounts of other nutrients, most insects are poor sources of calcium, and annelids, despite having less fat and high calcium values, have the same very variable nutritional contents, depending on the composition of the substrate where they are kept. The common practice of spraying or dipping insects with calcium supplements can provide inconstant or insufficient levels of calcium, can change their palatability and, if the insect is not consumed immediately, they can lose part or all the supplement (Bernard et al., 1997; Li et al., 2009). To combat this problem, there are already commercial diets rich in calcium for crickets that can be used to feed other insects. In addition, calcium supplements such as calcium carbonate can be added to the insect diet to increase its calcium content (Zwart et al., 1979; Strzelewicz et al., 1985; Allen et al., 1989; Finke, 2013).

Ultraviolet radiation

Ultraviolet (UV) radiation is a natural component of sunlight that is subdivided into three groups: the portion of UV radiation with the shortest wavelength (100–290 nm) is UVC radiation, the portion with an intermediate wavelength (290–320 nm) is UVB radiation, and the portion with the longest wavelength (320–400 nm) is UVA radiation (Adkins et al., 2003; Baines et al., 2016). UVB radiation enables the conversion of 7-dehydrocholesterol present in the skin into pre-vitamin D3 which, by thermal action, is converted into cholecalciferol (vitamin D3) (Baines et al., 2016). Pre-vitamin D_3 produced in excess is converted into inert photoproducts, lumisterol and tachysterol, by the action of UVB radiation and low-wavelength UVA radiation, *i.e.*, at wavelengths between 290 and 335 nm. This is a self-limiting process that prevents overproduction of vitamin D_3 (MacLaughlin et al., 1982, Webb et al., 1989). UVC radiation, which is normally completely filtered by the atmosphere, causes cell damage and therefore is not necessary and should not be added as an artificial source of radiation (Adkins et al., 2003).

Transmission of ultraviolet radiation

The skin acts as a barrier between the organism and the environment, protecting it from mechanical abrasion, microorganisms, water loss and UV radiation. Reptile skin, like the skin of many other vertebrates, has two main layers: the epidermis and the dermis (Subramaniam et al., 2018). The epidermis consists of a keratinised stratified epithelium and in reptiles of the order Squamata, which includes the leopard gecko, it is divided into several sub-layers: Oberhaütchen layer, β-keratin layer, α-keratin layer, intermediate zone, and germinal layer (basal stratum). The combination of the Oberhaütchen, β-keratin and α-keratin layers constitute the stratum corneum

(Subramaniam et al., 2018). The Oberhaütchen layer is the most superficial and hardest layer of the epidermis and is made up of dead flattened and anucleated cells, with keratin-rich cytoplasm. The β-keratin layers are composed of cells undergoing keratinisation, through the production of two types of keratin: α keratin, which is flexible, and β keratin, which is strong and hard, and only exists in reptiles. In this way, these layers become a hard and protective layer. The epidermis is in constant renewal, with the cells of the stratum germinativum as progenitor cells. After proliferation, the new keratinocytes migrate through the intermediate zone, until they reach the fully differentiated stratum corneum. Over time, these keratinocytes desquamate and will be replaced by the new generation of keratinocytes (Maderson et al., 1998; O'Malley, 2005; Subramaniam et al., 2018). Melanin, which is the pigment responsible for brown coloration, is found in epidermal cells and effectively absorbs UV radiation at wavelengths between 200 and 700 nm, which includes the UVB radiation responsible for the cutaneous production of vitamin D3 (Jablonski et al., 2000). Thus, the amount of melanin present in the epidermis will determine the amount of radiation that reaches the deeper layers of the skin (O'Malley, 2005; Baines et al., 2006; Rutland et al., 2019). It is in the membrane of the deepest cells of the epidermis that, due to the incidence of UV radiation, 7-dehydrocholesterol is converted into pre-vitamin D3 (Holick, 2004; Junqueira and Carneiro, 2013). UV radiation, before reaching these cells, crosses the most superficial layers of the epidermis. The epidermis of reptiles varies greatly from species to species both in thickness and in pigmentation. Microscopic observation of the skin of a green iguana (*Iguana iguana*), for example, has shown that it has a much higher level of keratinisation when compared to the skin of leopard geckos. In addition, the green iguana has the most pronounced β-keratin layer and very few melanocytes. This larger layer of keratin prevents the transmission of radiation through the epidermis and, consequently, when compared to the percentage of UV radiation passing through the skin of the two species, about four times more radiation will pass through the skin of the leopard gecko than the green iguana. The skin of lizard species that are exposed to higher intensities of radiation in their habitat shows a tendency to block more radiation, when compared with the skin of nocturnal, crepuscular species or those in areas with lower radiation intensity (Baines et al., 2006; Rutland et al., 2019). The dermis is the deepest layer of the skin, consisting of dense connective tissue, including adipocytes, blood vessels, lymphatic vessels, nerves, inflammatory cells and two types of pigment cells: xanthophores (yellow pigment) and melanophores (black pigment) (O'Malley, 2005; Szydłowski et al., 2015; Rutland et al., 2019). Season is also an important factor in transmitting radiation useful to produce vitamin D_3. A study performed in humans demonstrated that during June and July, the efficiency of vitamin D_3 production reached its peak, gradually decreasing after August, and ceasing almost completely between November and March (Webb et al., 1988). Latitude is another factor to consider, since it influences the zenith angle and, consequently, the intensity and period of exposure to solar radiation. In places closer to the poles, vitamin D_3 production ceases for six months of the year, while in places closer to the equator its synthesis occurs uninterruptedly throughout the year (Wacker and Holick, 2013a; Holick, 2018).

Benefits of ultraviolet radiation

Exposure to UV radiation brings several advantages to the organism (Juzeniene et al., 2012). Exposure to this radiation results in the activation of p53 which, in turn, regulates the expression of the gene encoding pro-opiomelanocortin. This is a polypeptide precursor, whose cleavage originates several bioactive products, among them, the melanocyte-stimulating hormone-α (α-MSH) and β-endorphin (Oren et al., 2007; Juzeniene et al., 2012). α-MSH is responsible for activating the production of melanin, which effectively absorbs UV radiation, protecting the skin from future exposure to this radiation (Catania et al., 2010). β-endorphin is the most abundant endogenous neurotransmitter in the blood and has analgesic effects and produces a sense of well-being (Bernard et al., 1991; Juzeniene et al., 2012; Fell et al., 2014; Veleva et al., 2018). In addition, UV radiation also promotes hyperkeratosis, thus providing greater protection (Bulat et al., 2011). UV radiation can also be used in the treatment and relief of symptoms of certain diseases. Heliotherapy (treatment with the use of sunlight) was already used in ancient Greece, Egypt and Rome for the treatment of various skin pathologies (Roelandts et al., 2002). There is evidence to suggest that exposure to UV radiation can decrease short-term pain in patients with fibromyalgia (Taylor et al., 2009). Access to this type of radiation is important to stimulate the natural behaviour of many species in captivity (Honkavaara et al., 2002). Several species regulate the period of exposure to radiation according to their physiological needs (Manning et al., 1997; Ferguson et al., 2003; Karsten et al., 2009; Oonincx et al., 2010).

Adverse effects of ultraviolet radiation

When exposure to UV radiation is excessive, several adverse effects may arise, mainly on the eyes and skin, both in cases of acute exposure and long-term exposure (Hathaway et al., 2016). In animals, there are reports of photodermatitis and keratoconjunctivitis in royal pythons and only photodermatitis in blue-tongued lizards, due to the long-term use of a UV lamp with excessive intensity. Other reptiles exposed to radiation with the same characteristics showed identical pathologies: a crested gecko (*Rhacodactylus ciliatus*), an albino Burmese python (*Python molurus bivittatus*), a tree boa of the genus *Candoia*, a bearded dragon (*Pogona vitticeps*), a snake of the species *Lampropeltis triangulum hondurensis*, and two Kenyan sand boas (*Érix colubrinus colubrinus*) (Gardiner et al., 2009). A study carried out on budgerigars (*Melopsittacus undulatus*) reported several adverse effects after exposure to UV radiation of different intensities. At lower intensities, effects such as weight loss, increased concentration of corticosterone in the blood and skin erythema were observed. At higher radiation intensities, animals developed corneal photokeratitis (Lupu et al., 2013). As in humans, skin cancers in captive reptiles may be related to exposure to UV radiation. It is possible that there is a relationship between radiation exposure and the incidence of squamous cell carcinomas and pigment cell neoplasms. A study with 69 reptiles suggested that heliophilic species have a higher incidence of this type of neoplasms when compared to nocturnal species. The same authors concluded, in another study, that around 60% of animals with pigment cell neoplasms had access to artificial UV radiation. However, these results are not conclusive and further studies are needed to understand this relationship in reptiles (Heckers et al., 2012, 2014).

Vitamin D and Calcium

At the end of the 19th century, after the industrial revolution, there was an outbreak of a disease characterised by skeletal deformities in children, known as rickets. Sir Edward Mellanby, to respond to this new outbreak, successfully used cod liver oil and the anti-rachitic properties of this substance were initially attributed to vitamin A (DeLuca et al., 2018). Vitamin D was discovered in 1922 by Elmer McCollum. After destroying the vitamin A present in cod liver oil, McCollum observed that it maintained its ability to cure rickets and concluded that the therapeutic properties of cod liver oil were not due to vitamin A, but to another substance which he called vitamin D (McCollum et al., 1922). Later, in 1925, Steenbock discovered that UV radiation had the ability to activate an inactive substance, giving rise to vitamin D (Steenbock et al., 1925).

Acquisition of vitamin D

Vitamin D can be acquired in two ways: through food and subsequent absorption in the gastrointestinal tract or through synthesis in the skin after exposure to UVB radiation (Hossein-Nezhad and Holick, 2013). In nature, vitamin D is available as ergocalciferol (vitamin D_2), whose precursor is ergosterol (provitamin D_2), or as cholecalciferol (vitamin D_3), whose precursor is 7-dehydrocholesterol (7-DHC) (pro-vitamin D_3) (Chen et al., 2010; Polzonetti et al., 2020). The difference between the two precursors is in the presence of an extra methyl group on carbon 24 of ergosterol (Chen et al., 2010; Engelking, 2015). Most authors consider that ergocalciferol (vitamin D_2) is found mainly in plants and fungi, and cholecalciferol (vitamin D_3) is found in products of animal origin (Chen et al., 2016; Polzonetti et al., 2020), though there is no consensus, since other authors consider that ergocalciferol is found only in fungi, while cholecalciferol is found in both animals and plants, albeit in small amounts (Wacker and Holick, 2013b; Göring et al., 2018). Vitamin D_3 can be obtained by eating foods that naturally contain this vitamin (cod liver oil, fatty fish such as salmon and tuna), mushrooms or plants exposed to UV radiation, foods fortified with vitamin D or through dietary supplements (Hossein-Nezhad and Holick, 2013). Alternatively, vitamin D_3 can be synthesised in the skin through exposure to UVB radiation. The skin of most vertebrates contains the precursor of vitamin D_3 (7-DHC) in the membranes of the deepest cells of the epidermis (Holick et al., 2014). Thus, when the skin is exposed to radiation with a wavelength between 290 and 320 nm (UVB radiation), 7-DHC is converted into pre-vitamin D_3 (Bunker et al., 1940; Chen et al., 2010; Watson, 2014). The energy of the photons, when incident on 7-DHC, causes a break in the bond between carbon 9 and carbon 10 of the molecule and an isomerization of the 5,7-diene, forming pre-vitamin D_3. This molecule is unstable and rapidly undergoes a rotation on the bond between carbon 5 and carbon 6 and a rearrangement of its hydrogen atoms, through a thermo-dependent process, giving rise to vitamin D_3 or cholecalciferol (Holick et al., 1995; Chen et al., 2010; Holick, 2018). After being produced in the skin, vitamin D_3 is transported to the liver where it undergoes hydroxylation at carbon 25, mediated by the enzyme 25-hydroxylase. 25-hydroxycholecalciferol, 25-hydroxyvitamin D_3 or calcidiol (25(OH)D_3) 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 form of this vitamin. In addition to its presence in the blood, it is stored in different places such as the liver, adipose tissue, and skeletal muscle tissue (Dahlback et al., 1988; Cline, 2012; Holick, 2014; Jones et al., 2018). To become biologically active, this form of vitamin D has to undergo a second hydroxylation, this time at carbon 1. This hydroxylation is mediated by the enzyme 1α-hydroxylase and gives rise to 1,25-dihydroxyvitamin D_3 (1,25(OH)2D_3), 1,25-dihydroxycholecalciferol or calcitriol, the active hormonal form of vitamin D_3 (Zehnder et al., 2002; Cline, 2012; Holick, 2014). This reaction occurs mainly in the kidneys (Zehnder et al., 2001, 2002; Larner et al., 2018). Vitamin D_2 or ergocalciferol can only be obtained through food. Like vitamin D_3, it undergoes two hydroxylations: the first in the liver and the second in different parts of the body, giving rise to 25-hydroxyergocalciferol or ercalcidiol and 1,25-dihydroxyergocalciferol or ercalcitriol, respectively (Holick, 2014; Dereje et al., 2017).

Vitamin D transport

Lipophilic steroid molecules, such as vitamin D, bind to plasma proteins in their blood transport. The main transport protein for vitamin D and its metabolites is vitamin D binding protein. This protein is responsible for about 80% of transport, while proteins such as albumin and lipoproteins, which are more abundant in the blood, have a less important role in this transport (Bouillon and Pauwels, 2017). Vitamin D binding protein, despite transporting all vitamin D metabolites, has a higher affinity for transporting calcidiol and a lower affinity for transporting calcitriol or vitamin D itself. In most species, this protein has a higher affinity for vitamin D_3 metabolites than for vitamin D_2 metabolites. In birds, the affinity of vitamin D binding protein for transporting vitamin D_3 metabolites is three to ten times greater than the affinity for transporting vitamin D_2 metabolites (Haddad et al., 1976; Bouillon et al., 1980; Marx et al., 1989; Bouillon and Pauwels, 2017).

Vitamin D functions

Vitamin D's main function is to maintain blood calcium and phosphorus levels within the values considered physiological and necessary for various metabolic functions, the regulation of gene expression and the success of bone metabolism (Fleet, 2018). It is estimated that vitamin D influences between 200 and 2000 genes, directly or indirectly, suggesting that an increase in vitamin D values can regulate the expression of genes related to oncological pathologies, autoimmune pathologies, and cardiovascular diseases which, in turn, are associated with a deficiency of this vitamin (Nagpal et al., 2005; Hossein-Nezhad and Holick, 2013). Vitamin D plays, together with parathyroid hormone (PTH), an important role in bone remodelling, affecting the metabolism of calcium and phosphorus (Carmeliet, 2018). When the calcium balance is normal or positive, vitamin D and PTH are responsible for keeping calcium levels within their normal range. However, when the calcium balance is negative, the parathyroid is stimulated, increasing PTH secretion. In turn, PTH stimulates the production of the active form of vitamin D, 1,25(OH)2D_3, through increased renal expression of 1α-hydroxylase. These two hormones promote bone remodelling, as they stimulate osteoblasts to produce cytokines that accelerate osteoclast maturation and, consequently, the release of calcium and phosphorus into circulation (Suda et al., 2015; Carmeliet, 2018; Nakamichi et al., 2018; Charoenngam et al., 2019). 1,25(OH)2D_3 stimulates the secretion of fibroblast growth factor (FGF23) in osteocytes. This hormone, unlike PTH, suppresses the activity of 1α-hydroxylase in the kidneys, reducing its production. The activity of the enzyme is also suppressed by the active form of vitamin D itself, which further suppresses PTH secretion in the parathyroid through its interaction with vitamin D receptors in the glands (Suda et al., 2015; Carmeliet, 2018; Nakamichi et al., 2018; Charoenngam et al., 2019). The increase in the concentration of 1,25(OH)2D_3 also promotes the expression of 24-hydroxylase, the enzyme responsible for the inactivation of vitamin D through the hydroxylation of carbon 24, culminating in the formation of calcitroic acid. The expression of this enzyme is suppressed by PTH (Tanaka and DeLuca, 1981; Holick, 2014). Both PTH and vitamin D stimulate renal calcium reabsorption. On the other hand, the increase in phosphorus blood concentrations stimulate the secretion of PTH and FGF23 which, consequently, increase its renal excretion. In addition, vitamin D plays a very important role in the absorption of calcium at the intestinal level, through two different mechanisms: through transcellular transport, active and saturable, which occurs mainly in the proximal small intestine (duodenum and jejunum), or through passive and non-saturable paracellular transport, which occurs with the same intensity throughout the entire intestine (Pansu, 1981). Vitamin D appears to play an active role in these two mechanisms. Without it, the body is only able to absorb 10 to 15% of the ingested calcium. However, the interaction of the active form of vitamin D with vitamin D receptors in the intestine can increase the efficiency of absorption to much higher values, 30% to 40% (Holick, 2007). In animals deficient in this vitamin, absorption efficiency can decrease by more than 75% (Pansu et al., 1983).

Photoisomerisation and photodegradation of vitamin D

The body has several vitamin D regulation mechanisms that prevent its excessive production (Chen, 2010). Pre-vitamin D produced in the skin can be converted into vitamin D_3 or other photoproducts such as tachysterol and lumisterol (Holick et al., 1981; MacLaughlin et al., 1982). In addition, the vitamin D_3 produced and accumulated in the skin is sensitive to radiation and is degraded when exposed, creating various photoproducts. A study showed that solar radiation in summer in Boston can degrade more than 50%, 75% and 95% after 30 minutes, one hour and three hours of exposure, respectively. The main photoproducts found were suprasterol 1, suprasterol 2 and 5,6-transvitamin D_3 (analogous molecule of vitamin D_3) (Webb et al., 1989). Thus, prolonged exposure to UV radiation does not continuously increase the plasma concentration of 25(OH)D_3 (Watson, 2014).

Vitamin D intoxication

Vitamin D in high doses can be toxic (O'Malley, 2008). However, there are no reported cases of pathologies derived from excess vitamin D caused by excessive exposure to radiation. This happens since both pre-vitamin D_3 and vitamin D_3 itself are susceptible to radiation, giving rise to various products that are inert or inactive in calcium homeostasis (Baines et al., 2006; Chen et al., 2010; Watson, 2014). Vitamin D intoxication caused by a natural diet is rare, and most cases described in reptiles are derived from an overdose of supplementation or an inadequate diet, as is the case of reptiles fed with dog or cat food containing high concentrations of vitamin D (Wallach, 1996; O'Malley, 2008; Penning, 2012; Boyer and Scott, 2019). In addition, intoxication can be caused acutely by the ingestion of cholecalciferol-based rodenticides or topical drugs for psoriasis, although this hypothesis is unlikely in captive reptiles (Cline, 2012; Dee and Hovda, 2012). Hypervitaminosis D can be difficult to diagnose, mainly due to the slow onset of clinical signs and, therefore, the prognosis can be very guarded (Cline, 2012). Excess vitamin D causes hypercalcemia and, consequently, mineralisation of the soft tissues of the gastrointestinal tract, muscles, kidneys, lungs, heart, and large vessels, which can usually be observed on radiographs or ultrasounds, and in the long term can cause bone malformations. These mineralisations can cause pain and organ dysfunction or failure (Wallach, 1996; Raiti and Garner, 2006; O'Malley, 2008; Cline, 2012; Penning, 2012; Watson, 2014; Boskey, 2018; Boyer and Scott, 2019). Currently there are no guidelines for the treatment of these pathologies in reptiles and therefore the treatment recommended for others is followed, based on glucocorticoids, calcitonin and, according to more recent studies, pamidronate (O'Malley, 2008; Boyer and Scott, 2019).

Vitamin D deficiency

In reptiles, the main consequence of hypovitaminosis D is metabolic bone disease (MBD) caused by nutritional secondary hyperparathyroidism (Watson, 2014). MBD is a term used to describe a set of diseases that affect the integrity and function of bones (Carmel and Johnson, 2017). The most common MBD in reptiles is secondary nutritional hyperparathyroidism (NSHP), whose main causes are prolonged calcium or vitamin D deficiency, an imbalance in the calcium-phosphorus ratio in the diet, inadequate exposure to UV radiation or the lack of adequate temperatures in the facilities (Carmel and Johnson, 2017; Lock, 2017). MBD is more common in turtles and lizards, as many of these animals have a diet with lower concentrations of vitamin D, while snakes have greater access to vitamin D through their diet (Carmel & Johnson, 2017). Decreased blood calcium or vitamin D concentrations and increased blood phosphorus concentrations increase PTH secretion, which in turn stimulates bone resorption. When deficiencies are prolonged, bone tissue destroyed during bone resorption is not recovered, causing a decrease in bone density (osteopenia) and weakening of the bones (Lock, 2017). The most common clinical signs include deviations in the spine and tail, swelling and stiffness of the hind limbs, bone fractures, bowed and soft long bones, deformed carapaces in turtles, difficulty in lifting the body or tail, swollen and soft jaw ("rubber jaw"), tremors, paresis or paralysis of the limbs, and lethargy (O'Malley, 2008; Penning, 2012; Lock, 2017). The recommended treatment includes supplementation with calcium and vitamin D, nutritional support, improvement of the conditions of the facilities, analgesia, and calcitonin (it is not recommended to administer calcitonin in animals with hypocalcemia or with clinical neurological signs) (Lock, 2017; Boyer and Scott, 2019). Prognosis varies with the severity of clinical signs, but lizards and crocodiles respond better to treatment than chelonians. The prognosis is good in cases without a visible decrease in bone radiopacity, whereas animals with paresis, paralysis or constipation have a worse prognosis. Good nutrition with adequate calcium supplementation and UV radiation is essential (Boyer and Scott, 2019).

Ultraviolet radiation and vitamin D in different species

Due to the great diversity of species and diverse environmental adaptations, studies have been carried out to understand the individual needs of each species (Vergneau-Grosset et al., 2020). Most vertebrate animals contain 7-dehydrocholesterol in the skin in sufficient concentrations to produce significant amounts of vitamin D_3 when exposed to UV radiation (Holick et al., 2014). A study carried out on leopard geckos (*Eublepharis macularius*) demonstrated that these animals, when exposed to short periods of UV radiation (2 h), can significantly increase the concentration of 25-hydroxyvitamin D_3. These data once again demonstrate that even nocturnal species can produce vitamin D_3 when exposed to UV radiation, even for short periods (Gould et al., 2018). Indeed, several species with less opportunity for exposure to sunlight have been shown to have evolved an adaptation mechanism that allows them to synthesise vitamin D_3 more efficiently (Carman et al., 2000; Baines et al., 2006; Ferguson et al., 2015; Rutland et al., 2019). One study compared the 7-dehydrocholesterol conversion capacity of the Texas spiny lizard (*Sceloporus olivaceous*) and the gecko (*Hemidactylus turcicus*). Though the former species is diurnal, and the latter is nocturnal, the nocturnal species was found to have a greater ability to convert 7-dehydrocholesterol (Carman et al., 2000).

Conclusions

The leopard gecko is one of the most popular pet lizard species, mostly due to its docile temperament, ease of maintenance and reproduction in captivity, its high longevity and small size, its beauty and diversity of colours and patterns. Since this species is frequently maintained in captivity, it is important to clearly understand the importance and its needs in terms of UV radiation, vitamin D and calcium for its normal behaviour and maintenance of fundamental physiological functions.

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Pregled stila života leopard gekona i važnost ultraljubčastog zračenja, vitamina D i kalcija

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Vrstu leopard gekon (*Eublepharis macularius*) prvi put je opisao 1854. britanski herpetolog Edward Blyth. Danas je to jedna od najpopularnijih vrsta guštera za kućnog ljubimca, uglavnom zbog njegovog mirnog temperamenta, jednostavnosti održavanja i reprodukcije u zatočeništvu, njegove dugovječnosti i male veličine te njegove ljepote i raznolikosti boja i uzoraka. Ultraljubičasto (UV) zračenje je važan regulator ponašanja. Vitamin D regulira i brojne osnovne fiziološke funkcije u kralježnjaka, uglavnom homeostazu kalcija. Zbog velike raznolikosti vrsta gmazova i različitih prilagodbi okolišu, važno je znati potrebe i prilagodbe svake vrste u svezi UV, vitamina D i kalcija. Ovaj rad pruža čitateljima pregled stila života leopard gekona i važnosti ultraljubičastog zračenja, vitamina D i kalcija za tu vrstu.

Ključne riječi: *ponašanje, Eublepharis macularius, habitat, život, dodatak prehrani*