



# Vitamin D metabolism in dogs and cats and its relation to diseases not associated with bone metabolism

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## Abstract

Due to the presence of receptors in the cells of numerous body tissues, vitamin D is associated with several physiological functions that go beyond calcium and phosphorus homeostasis and control of bone metabolism in the body. In humans, several studies have associated lower vitamin D concentrations with numerous diseases, such as cancer, heart disease, autoimmune diseases and infectious diseases, and also with an increase in the total mortality rate of the population. Recently, this nutrient started to gain importance in veterinary medicine, and several articles have shown a correlation between low vitamin D status and diseases unrelated to bone metabolism. The present review aims to highlight the recent publications that investigated this relationship, bringing the evidence that exists so far in dogs and cats.

## KEYWORDS

1,25(OH)<sub>2</sub>D, 25(OH)D, calcidiol, calcitriol, cholecalciferol, hypovitaminosis D

## 1 | INTRODUCTION

The effects of vitamin D as an important regulator of bone metabolism and calcium homeostasis have been well known for almost 100 years (McCollum, Simmonds, Becker, & Shipley, 1922). For a long time, this was believed to be the only role of this vitamin in the metabolism of humans and animals. However, after the discovery of vitamin D receptors (VDR) in various human immune cells (Provvedini, Tsoukas, Deftos, & Manolagas, 1983), research into the pleiotropic effects of vitamin D intensified, and it was discovered that cells of almost all body tissues express VDR. This receptor is believed to be directly or indirectly involved in the regulation of about 2,000 genes, which correspond to almost 10% of the human genome (Ramagopalan et al., 2010).

In human medicine, although the cause and effect relationship is not proven, the relationship between vitamin D status and diseases unrelated to bone metabolism has been extensively studied in recent years, and lower vitamin D concentrations has been associated with increased cancer incidence (Djurasinović et al., 2018; Yin

et al., 2013); cardiovascular diseases, such as myocardial infarction (Giovannucci, Liu, Hollis, & Rimm, 2008), stroke and heart failure (Chowdhury et al., 2012; Muscogiuri et al., 2017), as well as autoimmune diseases (e.g., diabetes mellitus type I) (Pludowski et al., 2013), multiple sclerosis (Martinelli et al., 2014), rheumatoid arthritis (Kerr et al., 2011) and infectious diseases (White, 2008).

The relationship of vitamin D and diseases has also been investigated in dogs and cats, and some studies found association between low vitamin D status and some types of cancer (Selting, Sharp, Ringold, Thamm, & Backus, 2016; Wakshlag et al., 2011; Weidner et al., 2017), congestive heart failure (Kraus et al., 2014; Osuga et al., 2015), gastrointestinal diseases (Allenspach, Rizzo, Jergens, & Chang, 2017; Gow et al., 2011; Lalor et al., 2014; Titmarsh, Gow, Kilpatrick, Cartwright, et al., 2015; Titmarsh, Gow, Kilpatrick, Sinclair, et al., 2015), acute pancreatitis (Kim et al., 2017), acute polyradiculoneuritis (Laws, Kathrani, Harcourt-Brown, Granger, & Rose, 2018), chronic kidney disease (Cortadellas, Fernandez del Palacio, Talavera, & Bayón, 2010; Galler et al., 2012; Gerber, Hässig, & Reusch, 2003; Gerber, Hauser, & Reusch, 2004; Parker, Harjes, et al., 2017) and

infectious diseases (Lalor et al., 2012; Rodriguez-Cortes et al., 2017; Rosa, Schoeman, Berry, Mellanby, & Dvir, 2013; Titmarsh, Lalor, et al., 2015). Although these studies do not prove a cause-effect relationship, they provide a basis for future studies to investigate possible positive effects of vitamin D supplementation on improving health status and reducing the risk of developing diseases. They also enable possible prognostic and potential therapeutic role of vitamin D supplementation for dogs and cats affected by different diseases, as already demonstrated in other species as humans (Correa Freitas et al., 2019; Dalbeni, Scaturro, Degan, Minuz, & Delva, 2014; Gotsman et al., 2012; Jamilian et al., 2019; Salehpour, Hosseini, Nazari, Hosseini, & Saharkhiz, 2019; Schleithoff et al., 2006).

The main objectives of this review were to summarize current knowledge about non-bone functions of vitamin D in cats and dogs as well as its role in cardiovascular disorders, infectious diseases, non-infectious inflammatory disorders, autoimmunity, cancer, chronic kidney disease and critical illness.

## 2 | VITAMIN D METABOLISM IN DOGS AND CATS

Dogs and cats, unlike other mammals such as sheep, cattle, horses, pigs, rats and humans, are not able to synthesize vitamin D in the skin through sun exposure (How, Hazewinkel, & Mol, 1994; Morris, 1999). Therefore, these species are dependent on the dietary intake of vitamin D. This can be explained by the evolutionary adaptation of these animals, as cats are considered strict carnivores and dogs carnio-omnivores adapted to the consumption of prey animals. These prey animals store vitamin D in the liver, so if liver is consumed regularly, vitamin D deficiency is not expected. If, for a long period, no prey is consumed, dogs and cats can use their vitamin D stores, because it is a fat-soluble vitamin therefore the body can store it. Due to this evolutionary adaptation, carnivores are also considered more resistant to vitamin D poisoning than omnivorous animals, due to their adaptation to ingestion of large amounts (Morris, 2002a). In other species, cutaneous biosynthesis of vitamin D occurs through the conversion of 7-dihydrocholesterol to cholecalciferol (vitamin D<sub>3</sub>) when the skin is exposed to ultraviolet B rays. Dogs and cats however have a low ability to perform this synthesis as a result of the high activity of the enzyme 7-dihydrocholesterol- $\Delta$ 7-reductase, which converts 7-dehydrocholesterol into cholesterol, reducing concentrations of this vitamin D precursor in the skin and preventing its conversion to cholecalciferol (How et al., 1994; Morris, 1999).

Vitamin D is presented in two forms in nature: cholecalciferol (vitamin D<sub>3</sub>), found in animal products and tissues, and ergocalciferol (vitamin D<sub>2</sub>), found in products of plant origin. It is worth emphasizing the nutritional peculiarity of felines regarding vitamin D metabolism. This species does not use ergocalciferol as efficiently as it does cholecalciferol (Morris, 2002b), unlike dogs, which can efficiently use both forms (Parker, Rudinsky, & Chew, 2017). This may perhaps be explained by the eating habits of these animals in the wild. Cats are considered strict carnivores, so their historical dietary intake of

ergocalciferol is close to null. In contrast, as dogs are considered omnivores, their intake of ergocalciferol should be higher than cats, and, consequently, they developed the ability to convert ergocalciferol into 25(OH)D in the liver.

After vitamin D intake and intestinal absorption, in bloodstream both cholecalciferol and/or ergocalciferol are transported by a glycoprotein, the vitamin D-binding protein (VDBP), to the liver where a hydroxylation occurs at carbon 25 by the action of a microsomal enzyme of the cytochrome P450 family (CYP450), called CYP2R1, resulting in 25-hydroxyvitamin D (25(OH)D), also called calcidiol. Depending on the vitamin D status, cholecalciferol can be stored in the body, especially in the adipose tissue; however, a small portion (approximately 16%) can be found in muscle tissue (Heaney, Horst, Cullen, & Armas, 2009). Due to the cholecalciferol storage capacity of the adipose tissue, it is hypothesized that obese animals may present a decrease in circulating concentrations of 25(OH)D caused by possible sequestration of cholecalciferol by adipose tissue because there are some published papers that have observed an association between lower vitamin D concentrations and obesity in humans (Bell et al., 1985; Buffington, Walker, Cowan, & Scruggs, 1993; Compston et al., 1981; Liel, Ulmer, Shary, Hollis, & Bell, 1988). However, this was not confirmed in a study developed by Hookey, Backus, and Wara (2018) in which there was no correlation between body fat percentage and serum 25(OH)D concentrations in dogs, as well as no effect of weight loss on vitamin D status.

25(OH)D, after its synthesis in the liver, is transported to the kidneys by VDBP, where it undergoes to another hydroxylation process by the enzyme 1 $\alpha$ -hydroxylase (CYP27B1), a mitochondrial protein of the CYP450 family, this time at carbon 1, forming 1,25-dihydroxyvitamin D (1,25(OH)<sub>2</sub>D), also called calcitriol, the main active metabolite of vitamin D. Although this metabolite is mainly formed in the proximal renal tubules, its synthesis can occur in any tissue composed of cells expressing 1 $\alpha$ -hydroxylase. According to Norman (2008), this enzyme has already been identified in several human tissues, such as prostate, breast, colon, immune cells, beta-pancreatic cells, parathyroid gland, placenta, brain, endothelial cells and keratinocytes. It was observed in humans that Both 25(OH)D and 1,25(OH)<sub>2</sub>D circulate through the bloodstream, predominantly bound to VDBP; however, a smaller amount circulates bound to albumin (10%–15%). Less than 1% of these circulating metabolites are in their free form (Jassil, Sharma, Bikle, & Wang, 2017).

Calcitriol has a pleiotropic effect on the body through the VDR receptor, a transcription factor that belongs to the family of nuclear hormone receptors. This metabolite has a much greater ability to bind to it when compared to 25(OH)D. According to Haddad (1979), in humans, the calcitriol binds approximately 500-fold faster to VDR than 25(OH)D. Calcitriol binds to a hydrophobic portion of VDR forming a transcriptional hormone-receptor complex. This complex is heterodimerized with the retinoic acid receptor (RXR), forming the 1,25(OH)<sub>2</sub>D-VDR-RXR heterodimer. This last substance interacts with a specific DNA sequence, called VDRE (vitamin D response element), composed of two sequences of repeated hexanucleotides, separated by 3 base pairs. This interaction promotes

a genetic transcription, which results in the induction or repression of the specific messenger RNA. For this to occur, the recruitment of complexes of co-regulatory proteins by the heterodimer is required. Gene induction or repression promotes changes in protein expression necessary to produce a biological response (McKenna & O'Malley, 2002; Rochel, Wurtz, Mitschler, Klaholz, & Moras, 2000).

The synthesis of  $1,25(\text{OH})_2\text{D}$  is primarily dependent on  $1\alpha$ -hydroxylase in the renal tubules and is regulated by plasma calcium concentrations, parathyroid hormone (PTH), fibroblast growth factor (FGF-23) and the *klotho* gene (Shimada et al., 2004). When circulating concentrations of ionized calcium decrease, there is an increase in the synthesis of PTH, which in turn stimulates the activity of  $1\alpha$ -hydroxylase in the proximal renal tubules, resulting in an increase in calcitriol synthesis. Both  $25(\text{OH})\text{D}$  and  $1,25(\text{OH})_2\text{D}$  are inactivated by the 24-hydroxylase enzyme, also present in the proximal renal tubules, and as a result, 24,25 dihydroxyvitamin D ( $24,25(\text{OH})_2\text{D}$ ) and 1, 24,25-trihydroxy vitamin D ( $1,24,25(\text{OH})_3\text{D}$ ) are formed (Christakos, Ajibade, Dhawan, Fechner, & Mady, 2012; de Brito Galvao, Nagode, Schenck, & Chew, 2013).

The 24-hydroxylase activity is stimulated by calcitriol, which is an important protective mechanism against hypercalcemia (de Brito Galvao et al., 2013). In addition, the activity of this enzyme is also stimulated by FGF-23, so when serum phosphorus concentrations are high there is an increase in the synthesis and secretion of FGF-23, a hypophosphatemic peptide produced by bone tissue, mainly by osteocytes (de Brito Galvao et al., 2013). This peptide plays an important role in the reduction of serum phosphorus through the stimulation of phosphorus renal excretion, as well as the reduction of the synthesis of calcitriol, since this metabolite promotes intestinal absorption of phosphorus. *Klotho*, a transmembrane protein, acts as a cofactor and is necessary for FGF-23 activity. It is essential for the binding of FGF-23 with its receptor (de Brito Galvao et al., 2013). Metabolites  $24,25(\text{OH})_2\text{D}$  and  $1,24,25(\text{OH})_3\text{D}$  could be excreted by urine and bile, mainly in the form of calcitic acid and  $1,25(\text{OH})_2\text{D}$ -lactone (Parker, Harjes, et al., 2017). The biological functions of these metabolites are not yet well elucidated, but  $24,25(\text{OH})_2\text{D}$  is believed to act in the bone mineralization process (Norman, Okamura, Bishop, & Henry, 2002).

Recently, a new vitamin D metabolite has been discovered in cats, a  $25(\text{OH})\text{D}$  epimer, identified as 3-epi- $25(\text{OH})\text{D}_3$  (Sprinkle, Hooper, & Backus, 2018), which was not identified in the serum and plasma of dogs by the authors. Epimerization is an important chemical process for the regulation of some steroid hormones, but the biological functions of 3-epi- $25(\text{OH})\text{D}_3$  have not yet been fully elucidated. Some studies have reported that this epimer can bind to VDR but has reduced biological activity when compared to calcitriol (Masuda et al., 2000). It has also been reported that 3-epi- $25(\text{OH})\text{D}_3$  shows the ability to suppress PTH secretion, but without other calcemic effects that calcitriol promotes (Rehan et al., 2002).

In the study by Sprinkle et al. (2018), the effect of increased vitamin D intake on serum concentrations of metabolites  $25(\text{OH})\text{D}$  and 3-epi- $25(\text{OH})\text{D}_3$  was investigated. In this study, eight cats consumed a diet containing 1.36 IU/g of cholecalciferol for over 3 years. After

this time, these animals began to consume a diet that contained 5.62 IU/g of cholecalciferol (4.13 times more than the previous diet) for 6 weeks. It is noteworthy that the two diets provided contained cholecalciferol levels between the adequate intake (0.224 IU/g of dry matter) and the safe upper limit (30 IU/g of dry matter) according to the NRC (2006). After increasing cholecalciferol intake, serum concentrations of  $25(\text{OH})\text{D}$  remained relatively constant while concentrations of 3-epi- $25(\text{OH})\text{D}_3$  increased. This metabolic pathway seems to be an important protective mechanism and may explain why cats are considered resistant to vitamin D intoxication (Sih, Morris, & Hickman, 2001; Sprinkle et al., 2018). This was demonstrated by Sih et al. (2001) that conducted a study with 57 cats which were fed with a diet with 63-fold higher amount of cholecalciferol than recommended by the NRC, and after 18 months, no signs of intoxication or impairment of renal function were observed by measuring biochemical markers, urinalysis and histological evaluation of renal tissue, although mean  $25(\text{OH})\text{D}$  plasma concentrations reached  $429.4 \pm 46.12$  ng/ml, about 3.47 times higher than mean  $25(\text{OH})\text{D}$  plasma concentrations of the control group ( $123.76 \pm 6.08$  ng/ml).

### 3 | GENETIC FACTORS THAT ALTER THE METABOLISM OF VITAMIN D

There are congenital abnormalities caused by genetic mutations that have an impact on vitamin D metabolism. Two autosomal recessive genetic defects have been identified in humans: vitamin D type I-dependent rickets (VDDR-I) and vitamin D type II-dependent rickets (VDDR-II), both have also been reported in dogs and cats (Geisen, Weber, & Hartmann, 2009; Godfrey, Anderson, Barber, & Hewison, 2005; Johnson, Church, Barton, & Wood, 1988; LeVine et al., 2009; Schreiner & Nagode, 2003; Tanner & Langley-Hobbs, 2005). The VDDR-I is caused by a mutation in the gene that encodes the  $1\alpha$ -hydroxylase enzyme on chromosome 12q13.3 and results in an inadequate conversion of calcidiol to calcitriol, so that the animals affected present normal  $25(\text{OH})\text{D}$  serum concentrations and low concentrations of  $1,25(\text{OH})_2\text{D}$ . The VDDR-II occurs as a result of a defect in the gene encoding VDR, resulting in high concentrations of calcitriol, hypocalcemia, secondary hyperparathyroidism, bone hypomineralization and, in some cases, alopecia (LeVine et al., 2009).

In a recent study developed by Teshima et al. (2019), the type 1B vitamin D-dependent rickets (VDDR-1B) was identified and reported for the first time linked to a genetic variant of *CYP2R1* in a cat. This anomaly resulted in low serum concentrations of ionized calcium and  $1,25(\text{OH})_2\text{D}$ , and radiographic examination revealed bone demineralization and abnormal growth plaques.

In cases of VDDR-I and VDDR-IB, therapy consists of calcitriol administration at a dose that varies according to the animal's response. Geisen et al. (2009) used doses ranging from 10 to 25 ng/kg PO q24h for treatment of VDDR-I in cats. Teshima et al. (2019) used doses up to 3.5 ng/kg PO q24h for VDDR-IB treatment in a cat. Calcium supplementation associated with calcitriol administration is recommended.

In cases of VDDR-II, it is more difficult to increase circulating calcium concentrations in response to calcitriol supplementation, so higher doses of this metabolite associated with calcium supplementation are used, yet animals with this congenital abnormality usually have persistent hypocalcemia (Godfrey et al., 2005; Levine et al., 2009). In the study conducted by Godfrey et al. (2005), a cat affected by VDDR-II received  $110 \text{ ng kg}^{-1} \text{ day}^{-1}$  of calcitriol supplementation for 5 months and  $3.410 \text{ mg kg}^{-1} \text{ day}^{-1}$  of calcium carbonate, and there was no variation in plasma calcium concentrations. In another study conducted by Levine et al. (2009), there was no increase in serum calcium concentrations in a dog with VDDR-II who received daily supplementation of  $64 \text{ ng/kg}$  calcitriol and  $64 \text{ mg/kg}$  calcium carbonate.

#### 4 | SERUM CONCENTRATIONS AND VITAMIN D NUTRITIONAL RECOMMENDATIONS FOR DOGS AND CATS

Despite not being the active form of vitamin D, calcidiol is the metabolite used to assess vitamin D status in humans and companion animals due to its longer biological half-life, which can range from 10 days to 3 weeks, thus reflecting the current vitamin D status (Vicchio et al., 1993). Furthermore, the measurement of calcitriol is more complicated because of its hydrophobic nature and very low concentrations (picomolar) in bloodstream that is about 1,000 times lower than the circulating concentrations of calcidiol (Souberbielle et al., 2015).

Regarding serum 25(OH)D concentrations, there is not any consensus about what could be considered sufficient for healthy dogs and cats. For humans, the Institute of Medicine (IM) considers concentrations of  $\geq 20 \text{ ng/ml}$  as sufficient, whereas the International Osteoporosis Foundation and the United States Endocrine Society (IOFUSES) consider sufficient serum concentrations of 25(OH)D  $\geq 30 \text{ ng/ml}$  (Stocklin & Eggersdorfer, 2013). However, these values have been widely questioned by experts (Stocklin & Eggersdorfer, 2013) because these classifications consider only the vitamin D function related to calcium and phosphorus homeostasis in the body. There are already clinical trials and epidemiological studies suggesting that 25(OH)D concentrations above  $30 \text{ ng/ml}$  bring health benefits (Heaney, 2013; Hollis, 2011; Trivedi, Doll, & Khaw, 2003) and that concentrations close to  $40 \text{ ng/ml}$  are associated with maintenance of cardiovascular health, reduced risk of general mortality and prevention of colorectal cancer, without implying a risk of developing hypercalcemia (Bischoff-Ferrari et al., 2010). In addition, according to Deluca (2008), concentrations of 25(OH)D between  $75$  and  $90 \text{ ng/ml}$  seem to be beneficial in protecting against some degenerative diseases.

Selting et al. (2016) investigated 25(OH)D ideal serum concentrations for dogs. For this, the authors used the approach that determined as sufficient the lowest concentrations necessary to suppress PTH synthesis. Based on this approach, they recommended 25(OH)D serum concentrations between  $100$  and  $120 \text{ ng/ml}$  (measured by

chemiluminescent immunoassay), well above recommendations for humans. However, these values cannot be extrapolated to the general canine population, as laboratories use varied methodologies to measure 25(OH)D concentrations, and it can impact the results.

Given the lack of information regarding to the adequate levels of vitamin D for adult dogs and cats, the recommendations of the NRC are based on studies that determined the appropriate minimum levels for growing puppies, and these appropriate levels were based on non-occurrence of bone abnormalities so at that time point they did not take into account potential other functions of vitamin D in the organism. Therefore,  $110 \text{ IU}$  of cholecalciferol per  $1,000 \text{ kcal}$  of metabolizable energy (ME) is considered suitable for dogs and the safe upper limit is  $800 \text{ IU}/1,000 \text{ kcal}$  of ME. For cats, a content of  $56 \text{ IU}/1,000 \text{ kcal}$  of ME is considered appropriate and the safe upper limit is  $7,520 \text{ IU}/1,000 \text{ kcal}$  of ME. FEDIAF recommends for adult dogs  $159 \text{ IU}$  and  $138 \text{ IU}/1,000 \text{ kcal}$  of ME considering daily energy requirement of  $95 \text{ kcal} \times \text{kg body weight}^{0.75}$  and  $110 \text{ kcal} \times \text{kg body weight}^{0.75}$  respectively. For adult cats, the minimum cholecalciferol recommendations established by FEDIAF (2018) are  $83.3$  and  $62.2 \text{ IU}/1,000 \text{ kcal}$ , taking into account daily energy requirement of  $75 \times \text{kg}^{0.67}$  and  $100 \times \text{kg}^{0.67}$  respectively. FEDIAF does not indicate which study was considered to determine these recommendations, but states that studies of renowned researchers were taken into account. The safe upper limits for dogs and cats are the same as those recommended by the NRC.

It is not known whether these amounts of cholecalciferol recommended are enough for the animals to reach serum concentrations of 25(OH)D between  $100$  and  $120 \text{ ng/ml}$ , which were considered ideal for dogs (Selting et al., 2016). Sharp, Selting, and Ringold (2015) measured serum 25(OH)D concentrations by chemiluminescence immunoassay in  $292$  healthy dogs consuming commercial diets only, and the serum concentrations ranged from  $16.9$  to  $249.2 \text{ ng/ml}$  with a median of  $67.9 \text{ ng/ml}$ . However, there was no analysis of vitamin D content in the diets offered to the animals in that study; therefore, it was not possible to conclude whether the minimum recommended levels of vitamin D are insufficient to reach serum concentrations of 25(OH)D above  $100 \text{ ng/ml}$  since these diets might not be meeting the minimum level recommended.

In addition, in that study, the animals were not submitted to biochemical tests to investigate possible subclinical abnormalities, which could influence serum 25(OH)D concentrations. The animals were considered healthy only based on the absence of clinical signs and changes in the physical examination. Thus, some animals may have had diseases that could result in decreased vitamin D status, and this may lead to an underestimation of the potential of diets to modulate 25(OH)D concentrations.

To date, only one article has been published evaluating cholecalciferol concentrations in commercial dog foods (Kritikos et al., 2018). In this study, conducted in Canada, the cholecalciferol concentrations were determined by high-performance liquid chromatography-tandem mass spectrometry in samples from  $81$  commercial foods ( $72$  dry and  $9$  wet foods) provided by dog owners. Only one food presented cholecalciferol concentrations below the

minimum recommendation by the NRC, and the average content was 428 IU/1,000 kcal of ME. As there was no measurement of 25(OH)D concentrations in animals that consumed these diets, it remained inconclusive whether the recommended minimum levels of cholecalciferol by the NRC, which were met in 80/81 feeds evaluated, are sufficient to reach serum 25(OH)D concentrations above 100 ng/ml. Therefore, further research is needed to investigate the minimum vitamin D content required in the diet so dogs can maintain 25(OH)D serum concentrations between 100–120 ng/ml.

## 5 | VITAMIN D CONCENTRATIONS IN VARIOUS DISEASES

The Table 1 shows the serum/plasma 25(OH)D concentrations observed in dogs and cats with different diseases, as well as those observed in healthy animals (control groups) in all studies that evaluating vitamin D status in dogs and cats, and the results observed in each study.

### 5.1 | Vitamin D and gastrointestinal diseases

In dogs, the relationship between vitamin D and gastrointestinal diseases has been studied in recent years. Gow et al. (2011) observed that serum 25(OH)D levels were lower in dogs with inflammatory bowel disease (IBD) and hypoalbuminemia than in healthy dogs, dogs hospitalized for causes unrelated to IBD, and dogs with IBD with albumin levels within the reference range. In a second study developed by Titmarsh, Gow, Kilpatrick, Cartwright, et al. (2015), 39 dogs with histopathological diagnosis of chronic enteropathy were used, and a negative association was observed between serum concentrations of 25(OH)D and markers of gastrointestinal and systemic inflammation, such as monocyte and eosinophil counts, duodenal histopathological parameters and serum concentrations of interleukins (IL) 2 and IL-8.

Two studies evaluated the relationship between vitamin D status at the time of diagnosis of enteropathies and the lifespan of these animals, and both observed that 25(OH)D may be considered a predictor of clinical outcome. Titmarsh, Gow, Kilpatrick, Sinclair, et al. (2015) developed a study with 41 dogs diagnosed with chronic enteropathy and observed lower concentrations of 25(OH)D at the time of diagnosis in dogs that died or were euthanized as a result of this disease when compared to dogs that were alive or died due to causes unrelated to the enteropathy. In a more recent study by Allenspach et al. (2017), 43 dogs diagnosed with protein-losing enteropathy (PLE) were evaluated and they were divided into two groups: (a) a group that died up to 4 months after diagnosis (called as negative group,  $n = 22$ ) and (b) a group of dogs that were alive or who died from other causes unrelated to PLE until 1 year after diagnosis (group with positive result,  $n = 21$ ). It was observed that serum 25(OH)D concentrations were lower in dogs in the negative group. In cats, patients with IBD or small-bowel lymphoma had lower serum

concentrations of 25(OH)D than healthy animals and animals hospitalized for other causes (Lalor et al., 2014). None of these studies mentioned have monitored vitamin D status of animals prior to the disease development.

In humans, it has also been shown that patients with IBD have lower concentrations of 25(OH)D when compared to healthy individuals after dietary intake and seasonal variation adjusting (McCarthy et al., 2005). There are also studies which demonstrated that 25(OH)D may be a biomarker of IBD severity (Blanck & Aberra, 2013; Joseph, George, Pulimood, Seshadri, & Chacko, 2009; Ulitsky et al., 2011). The aetiology of IBD has not yet been fully elucidated, but it is believed that the deregulation of the immune response to commensal intestinal bacteria may be involved in its pathogenesis (Limketkai, Bechtold, & Nguyen, 2016). Several studies have demonstrated the implication of vitamin D in immune system regulation in humans (Baeke, Gysemans, Korf, & Mathieu, 2010; Booth et al., 2016; Hewison, 2010; Jeng et al., 2009; Liu et al., 2006; Vanherwegen, Gysemans, & Mathieu, 2017) and in dogs (Jaffey Amorim, & DeClue, 2017, 2018). The 1,25(OH)<sub>2</sub>D exerts its metabolic effect by means of hormonal signalling after activation of its nuclear receptor (VDR) in cells that express it, and subsequently, transcription factors that have regulatory effect on the rate of gene transcription are activated (through RNA polymerase II), including those involved in immune function (White, 2012).

Besides its expression in a variety of tissues, especially in human intestinal cells, the VDR is also present in T cells, neutrophils to antigen-presenting cells (macrophages and dendritic cells), that is signalling of vitamin D can influence both the system immuno-innate as the adaptive (White, 2012). Regarding the innate immune system, 1,25(OH)<sub>2</sub>D<sub>3</sub> can directly influence monocytes and macrophages to increase their proliferation, IL-1 production and synthesis of cathelicidin (antimicrobial defensin) and thus contribute to the innate response to bacteria. In dendritic cells, it reduces the expression of MHC II, CD40, CD80, CD86 and IL-12 and increases the expression of IL-10, inducing a tolerogenic response (Mora, Iwata, & Von Andrian, 2008).

In the cells of the adaptive system, 1,25(OH)<sub>2</sub>D<sub>3</sub> reduces the production of IL-2, IL-17 (much related to crohn disease in humans) (Miossec, 2009; Yen et al., 2006) and INF $\gamma$ . It also attenuates the proliferation of cell types such as CD4<sup>+</sup> and CD8<sup>+</sup> (Mora et al., 2008). Finally, this may still have a positive effect on the expression of FOXP3 and the formation of induced regulatory T cells (Mora et al., 2008). Although VDR is widely expressed in all tissues in dogs, the colon of healthy dogs is the only place where its expression is weak, unlike humans and other species (Cartwright et al., 2018). In humans and mice used as an experimental model for ulcerative colitis, the VDR expression was negatively related to colonic inflammation; however, in studies carried out by Cartwright et al. (2018) no changes in VDR expression were observed in dogs with inflammatory bowel disease. The authors also observed that the expression of VDR was not correlated with the presence of inflammation, which, may confer an advantage for the treatment of these animals with vitamin D replacement.

**TABLE 1** Serum/plasma concentrations of 25(OH)D in dogs and cats with various diseases and in healthy animals observed in different studies

Reference	Specie	Disease	25(OH)D (ng/ml) disease group	25(OH)D (ng/ml) control group	Material	Methodology	Observed results
Wakshlag et al. (2011)	Dog	Cutaneous mast cell tumours	41.6 ± 12 <sup>a</sup>	48 ± 14 <sup>a</sup>	Serum	Radioimmunoassay	25(OH)D serum concentrations in dogs with mastocytoma were lower than those of healthy dogs
Selting et al. (2016)	Dog	Splenic hemangiosarcoma	49.2 (19.4–91.8) <sup>b</sup>	68.9 (9.5–249) <sup>b</sup>	Serum	Chemiluminescence immunoassay	An increased relative risk for cancer was observed as serum 25(OH)D levels decreased. The relative risk of cancer for 25(OH)D below 40 ng/ml of all-cancer group and of splenic hemangiosarcoma group were 3.9 and 4.1, respectively, compared with that of the control group
Willcox et al. (2016)	Dog	Osteosarcoma	34.95 ± 11.54 <sup>a</sup>	33.85 ± 10.27 <sup>a</sup>	Serum	Liquid chromatography–tandem mass spectrometry	No difference in serum concentrations of 25(OH)D in dogs affected by osteosarcoma, compared with a control group matched for age and body weight
Weidner et al. (2017)	Dog	Osteosarcoma Lymphoma Mast cell tumour	41.9 ± 20.6 <sup>a</sup> 41.1 ± 14.4 <sup>a</sup> 44.9 ± 12.3 <sup>a</sup>	51.3 ± 16.7 <sup>b</sup> 51.3 ± 16.7 <sup>a</sup> 51.3 ± 16.7 <sup>a</sup>	Plasma	Radioimmunoassay	The relationship between cancer and a change in vitamin D metabolism was suggested. In cancer patients, plasma 25(OH)D concentrations increased as ionized calcium increased, whereas in healthy dogs, plasma 25(OH)D concentrations decreased with the increase of ionized calcium concentrations
Gow et al. (2011)	Dog	Protein-losing enteropathy Inflammatory bowel disease Hospitalized patients with non-gastrointestinal illness	5.7 <sup>b</sup> 28.6 <sup>b</sup> 26.9 <sup>b</sup>	30.8 <sup>b</sup> 30.8 <sup>b</sup> 30.8 <sup>b</sup>	Serum	Radioimmunoassay	Serum 25(OH)D levels were lower in dogs with protein-losing enteropathy than in healthy dogs, dogs hospitalized for causes unrelated to inflammatory bowel disease, and dogs with inflammatory bowel disease with albumin levels within reference range
Titmarsh, Gow, Kilpatrick, Sinclair, et al. (2015)	Dog	Chronic enteropathy (survivors) Chronic enteropathy (non-survivors)	24.90 (15.63–39.45) <sup>c</sup> 4.3 (1.6–17.0) <sup>c</sup>	— —	Serum	Radioimmunoassay	Lower serum 25(OH)D concentrations at the time of diagnosis in dogs that died or were euthanized as a result of this disease than dogs who were alive or died due to causes unrelated to this disease
Allenspach et al. (2017)	Dog	Protein-losing enteropathy (negative outcome) Protein-losing enteropathy (positive outcome)	6.6 (0–26.4) <sup>b</sup> 14.8 (2.4–32.4) <sup>b</sup>	— —	Serum	Radioimmunoassay	Serum 25(OH)D concentrations were lower in dogs that died up to 4 months after diagnosis (negative outcome group) than dogs that were alive or who died from other causes unrelated to protein-losing enteropathy until 1 year after diagnosis (positive outcome group)

(Continues)

TABLE 1 (Continued)

Reference	Specie	Disease	25(OH)D (ng/ml) disease group	25(OH)D (ng/ml) control group	Material	Methodology	Observed results
Kraus et al. (2014)	Dog	Congestive heart failure	40 ± 16.8 <sup>a</sup>	49.2 ± 17.6 <sup>a</sup>	Serum	Radioimmunoassay	Dogs with congestive heart failure presented lower 25(OH)D concentrations than healthy dogs. It was demonstrated an association between low 25(OH)D concentrations and worse clinical outcomes
Osuga et al. (2015)	Dog	Chronic valvular heart disease stage B1 Chronic valvular heart disease stage B2 Chronic valvular heart disease stage C/D	21.76 (13.4–71.2) <sup>b</sup> 14.32 (1.96–68.8) <sup>b</sup> 5.24 (1.96–23.2) <sup>b</sup>	— — —	Serum	Enzyme-linked immunosorbent assay	It was observed an association between vitamin D and degree of cardiac remodeling, in which 25(OH)D concentrations were significantly lower in the B2 and C/D stages than B1 stage of congestive heart failure
Kim et al. (2017)	Dog	Acute pancreatitis (survivors) Acute pancreatitis (non-survivors)	24 <sup>b,d</sup> 10 <sup>b,d</sup>	40 <sup>b,d</sup> 40 <sup>b,d</sup>	Serum	Electrochemiluminescence immunoassay	25(OH)D concentrations were lower in dogs with acute pancreatitis than in healthy dogs. In addition, dogs which survived to acute pancreatitis had 25(OH)D concentrations higher than those of dogs who died due to this disease before the beginning of treatment
Jaffey, Amorim, et al. (2018)	Dog	Critically ill patients (survivors) Critically ill patients (non-survivors) Critically ill patients with sepsis	32 (21–40) <sup>c,d</sup> 20 (13–29) <sup>c,d</sup> 22 (17–34) <sup>c,d</sup>	43 (20–54) <sup>c,d</sup> 43 (20–54) <sup>c,d</sup> 43 (20–54) <sup>c,d</sup>	Serum	High-performance liquid chromatography	Critically ill dogs or dogs with sepsis had lower serum 25(OH)D concentrations compared to healthy dogs. Besides, 25(OH)D was considered a good predictor of survival for dogs in the intensive care unit and 30 days after discharge and it presented a correlation with illness severity
Rosa et al. (2013)	Dog	Spirocerosis neoplastic patients Spirocerosis non-neoplastic patients	12.28 (5.88–24.88) <sup>b</sup> 21.08 (7.64–51.88) <sup>b</sup>	29.84 (14.96–52.2) <sup>b</sup> 29.84 (14.96–52.2) <sup>b</sup>	Serum	High-performance liquid chromatography	Serum 25(OH)D concentrations were lower in dogs with neoplastic and non-neoplastic spirocerosis compared to healthy animals
Rodriguez-Cortes et al. (2017)	Dog	Visceral leishmaniasis (symptomatic dogs) Visceral leishmaniasis (asymptomatic dogs)	19.6 (10.62–25.14) <sup>c</sup> 29.6 (24.64–40.03) <sup>c</sup>	31.8 (25.95–34.65) <sup>c</sup> 31.8 (25.95–34.65) <sup>c</sup>	Serum	Enzyme-linked immunosorbent assay	Lower 25(OH)D concentrations were observed in symptomatic dogs than non-infected and asymptomatic patients. Besides, an association between lower vitamin D concentrations and progression of visceral leishmaniasis was observed in dogs

(Continues)

TABLE 1 (Continued)

Reference	Specie	Disease	25(OH)D (ng/ml) disease group	25(OH)D (ng/ml) control group	Material	Methodology	Observed results
O'Brien et al. (2018)	Dog	Blastomycosis	31.6 (13.2–50) <sup>b</sup>	52.8 (32.4–83.6) <sup>b</sup>	Serum	Radioimmunoassay	Dogs naturally affected by blastomycosis had lower 25(OH)D concentrations than healthy dogs. Also an association between 25(OH)D concentrations and neutrophil count, partial carbon dioxide pressure, and the bone and cutaneous involvement of the illness was observed
Gerber et al. (2003)	Dog	Chronic renal failure Acute renal failure	33.6 ± 24 <sup>a</sup> 52 ± 32.8 <sup>a</sup>	106.8 ± 38.8 <sup>a</sup> 106.8 ± 38.9 <sup>a</sup>	Serum	Protein-binding assay	Dogs with chronic renal failure or acute renal failure presented lower 25(OH)D concentrations than healthy dogs
Gerber et al. (2004)	Dog	Hypercalcemia and chronic renal failure Hypercalcemia and lymphoma Hypercalcemia and primary hyperparathyroidism	26.8 (14–73.6) <sup>b</sup> 40.6 (25.6–116.4) <sup>b</sup> 36.4 (26.4–119.2) <sup>b</sup>	122.6 (19.2–140) <sup>b</sup> 122.6 (19.2–140) <sup>b</sup> 122.6 (19.2–140) <sup>b</sup>	Serum	Protein-binding assay	Dogs with lymphoma, primary hyperparathyroidism and chronic renal failure presented lower 25(OH)D concentrations compared to healthy dogs
Galler et al. (2012)	Dog	Chronic kidney disease	19.2 ± 14.1 <sup>a</sup>	40.7 ± 16.5 <sup>a</sup>	Plasma	Liquid chromatography tandem mass spectrophotometry	Dogs with chronic kidney disease presented lower 25(OH)D concentrations compared to healthy dogs
Parker, Harjes, et al. (2017)	Dog	Chronic kidney disease (total n) Chronic kidney disease (stage I) Chronic kidney disease (stage II) Chronic kidney disease (stage III) Chronic kidney disease (stage IV)	48.2 (3.5–95.8) <sup>b</sup> 53.7 (32.6–87.3) <sup>b</sup> 55.7 (34.5–93.5) <sup>b</sup> 42.7 (3.5–95.8) <sup>b</sup> 25.0 (5.7–52.5) <sup>b</sup>	75.1 (50.4–97.9) <sup>b</sup> 75.1 (50.4–97.9) <sup>b</sup> 75.1 (50.4–97.9) <sup>b</sup> 75.1 (50.4–97.9) <sup>b</sup> 75.1 (50.4–97.9) <sup>b</sup>	Serum	Radioimmunoassay	25(OH)D concentrations were lower in dogs with stages 3 and 4 of chronic kidney disease, compared to healthy dogs, but not in dogs with stages 1 and 2 of chronic kidney disease. Besides, 25(OH)D was negatively correlated with parathyroid hormone, fibroblast growth factor-23, and phosphorus concentrations
Lalor et al. (2014)	Cat	Inflammatory bowel disease and intestinal small cell lymphoma Hospitalized cats with non-gastrointestinal diseases	12.7 <sup>b</sup> 33.8 <sup>b</sup>	45.1 <sup>b</sup> 45.1 <sup>b</sup>	Serum	Liquid chromatography-tandem mass spectrometry	Patients with inflammatory bowel disease or small-bowel lymphoma had lower serum concentrations of 25(OH)D than healthy animals and animals hospitalized for other causes
Titmarsh, Kilpatrick, et al. (2015)	Cat	Hospitalized patients (alive) Hospitalized patients (dead)	38.5 (1.7–81.6) <sup>b</sup> 22.9 (8.7–97.1) <sup>b</sup>	– –	Serum	Liquid chromatography-tandem mass spectrophotometry	Although 25(OH)D concentrations were higher in cats that were alive after 30 days compared to cats that died, it was not considered a predictor of mortality as there was not a linear regression. However, when 25(OH)D was represented as a categorical variable, cats with 25(OH)D concentration in the lower tercile had a higher risk of mortality than cats in the middle tercile reference category

(Continues)



TABLE 1 (Continued)

Reference	Specie	Disease	25(OH)D (ng/ml) disease group	25(OH)D (ng/ml) control group	Material	Methodology	Observed results
Lalor et al. (2012)	Cat	Mycobacteria	22.15 (9.7–54.8) <sup>b</sup>	49 (22.9–83.1) <sup>b</sup>	Serum	High-performance liquid chromatography	25(OH)D concentrations were lower in cats affected by mycobacteria than those found in healthy animals
Titmarsh, Lalor, et al. (2015)	Cat	Feline immunodeficiency virus Hospitalized cats	32.10 (5.0–62.4) <sup>b</sup> 30.9 (7.1–82.40) <sup>b</sup>	44.7 (14.9–61.0) <sup>b</sup> 44.7 (14.9–61.0) <sup>b</sup>	Serum	High-performance liquid chromatography	Cats infected with feline immunodeficiency virus and hospitalized cats presented lower 25(OH)D concentrations than healthy cats

<sup>a</sup>Value reported is mean  $\pm$  SD.

<sup>b</sup>Value reported is median (range) or median.

<sup>c</sup>Value reported is median (interquartile range).

<sup>d</sup>Approximate value, because original article did not provide the values, only showed the results through graphs.

Until this moment, it is not known whether lower vitamin D concentrations are the cause or only a consequence of intestinal diseases. It is believed that a decrease in vitamin D intake or absorption, caused by the disease, may be associated. However, results reported by Gow et al. (2011) and Lalor et al. (2014) suggested that other mechanisms are involved, since patients hospitalized due to other causes unrelated to enteropathies, which could also cause a reduction in appetite, presented higher serum 25(OH)D concentrations than the patients with enteropathy. In addition, inflammation of gastrointestinal mucosa can impair the absorption of vitamin D, contributing to a decrease in its serum status. However, there is evidence that low concentrations of 25(OH)D may influence the development of the inflammatory bowel process (Li, Chen, & Du, 2015). It is known that 25(OH)D concentrations are negatively associated with inflammatory mediators such as IL-8 (Titmarsh, Gow, Kilpatrick, Cartwright, et al., 2015). This cytokine plays an important role in the initiation and maintenance of IBD in humans through the recruitment of neutrophils into the inflamed gastrointestinal tract (Mazzucchelli et al., 1994). As previously mentioned, Titmarsh, Gow, Kilpatrick, Cartwright, et al. (2015) demonstrated a negative association between serum concentrations of 25(OH)D and circulating IL-8, a fact that could support this hypothesis. In another study, Hidaka, Wakabayashi, Takeda, and Fukuzawa (2013) observed anti-inflammatory effects of vitamin D by decreasing IL-8 production in intestinal cell culture.

Studies with experimental models of IBD have shown anti-inflammatory effects of vitamin D. In one study with mice, 1,25(OH)<sub>2</sub>D inhibited several genes involved in regulating the production and signalling of tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) (Zhu, Mahon, Froicu, & Cantorna, 2005). In another study conducted by Froicu and Cantorna (2007), genetically modified mice that did not have VDR, when subjected to induced gastrointestinal inflammation, produced higher amounts of pro-inflammatory cytokines when compared to wild mice. These results suggest that vitamin D plays an important role in regulating the gastrointestinal inflammatory process; however, caution is recommended when extrapolating these findings to dogs and cats.

In humans, Yang et al. (2013) did not observe changes in the concentrations of inflammatory mediators after patients with IBD received vitamin D supplementation, but it is not known if the required dose should be higher. Therefore, the role of vitamin D supplementation in the development and treatment of IBD in dogs and cats needs further studies to be elucidated.

## 5.2 | Vitamin D and cancer

The relationship between vitamin D and cancer has been widely studied in recent years in humans. As a result, epidemiological data showed that 25(OH)D serum concentrations above 40 ng/ml correlated with reduced risk of breast, colon and rectal cancer (Welsh, 2012). The evidence on the relationship between vitamin

D status and cancer risk is stronger for the three types of cancer mentioned above; however, other types of cancer, such as endometrial, bladder, brain, oesophagus, gallbladder, kidneys, lungs, ovarian, pancreas, prostate and stomach cancer, may also be vitamin D sensitive (Welsh, 2012). Still according to this author, vitamin D has been increasingly associated with cancer prevention in clinical studies, studies with experimental animal models and epidemiological studies. In addition, concentrations of 25(OH)D are inversely associated with total cancer incidence and mortality (Yin et al., 2013).

After Russell, Rassnick, Erb, Vaughan, and McDonough (2010) demonstrated the presence of VDR in canine mastocytoma neoplastic cells, some studies which investigated the relationship between vitamin D and cancer in dogs were published. Wakshlag et al. (2011) evaluated the relationship between serum concentrations of 25(OH)D and mastocytoma in Labrador Retrievers. In total, 33 animals affected by this disease were evaluated and compared with a control group that was composed of 54 healthy animals, and lower serum concentrations 25(OH)D were observed in dogs with mastocytoma.

In another study conducted by Selting et al. (2016), the 25(OH)D serum concentrations from a group of healthy dogs (control group;  $n = 282$ ) were compared to 25(OH)D serum concentrations from a group of dogs with splenic malignancies (all-cancer group;  $n = 40$ ) and with another group of dogs with benign neoplasms (benign group;  $n = 22$ ). The all-cancer group was divided into two groups: one group of dogs with splenic hemangiosarcoma (SHA group;  $n = 31$ ) and another group of dogs with other splenic malignancies ( $n = 9$ ), and these two groups were also compared with the control group. The control group presented higher 25(OH)D serum concentrations when compared to all other groups. In addition, an increased relative risk (RR) for cancer was observed as serum 25(OH)D levels decreased; therefore, the RR of cancer for 25(OH)D below 40 ng/ml of all-cancer group and of HAS group were 3.9 and 4.1, respectively, compared with that of the control group. In contrast, Willcox, Hammett-Stabler, and Hauck (2016) found no difference in serum concentrations of 25(OH)D in 20 dogs affected by osteosarcoma, compared with a control group matched for age and body weight.

Studies have demonstrated *in vitro* antineoplastic effects of calcitriol for various types of canine tumours, including osteosarcoma (Barroga, Kadosawa, Okumura, & Fujinaga, 1999), squamous cell carcinoma (Kunakornsawat et al., 2001), prostatic epithelial tumour (Kunakornsawat et al., 2004), anal sac adenocarcinoma (Kunakornsawat et al., 2002), mammary gland cancer (Rassnick et al., 2008) and mastocytoma (Malone et al., 2010). These findings reinforce the hypothesis that low concentrations of vitamin D may increase the risk of developing cancer. Besides, the antineoplastic effects of 1,25(OH)<sub>2</sub>D have also been demonstrated *in vivo* studies with dogs. Malone et al. (2010) demonstrated that 1,25(OH)<sub>2</sub>D, used alone for treatment of mastocytoma, induced remission in 4 (one complete remission, when dosing 2.25 µg calcitriol kg<sup>-1</sup> week<sup>-1</sup>, and three partial remissions when dosing 1.5 µg calcitriol kg<sup>-1</sup> week<sup>-1</sup>) out of the 10 dogs who participated of the

experiment. Rassnick et al. (2008) also observed synergistic effect *in vivo* of 1,25(OH)<sub>2</sub>D with chemotherapeutic drug cisplatin.

Several *in vitro* studies with human tumour cell lines have investigated the different mechanisms by which calcitriol plays anti-cancer effects. These mechanisms are described below.

## 5.2.1 | Control of cell differentiation and growth

### *Cell-cycle regulators*

Regarding the differentiation and cell growth regulation, it is possible that some components of the cell cycle can be directly or indirectly influenced by 1,25(OH)<sub>2</sub>D, such as inhibition of positive controls (cyclins and kinases dependent on cyclin) and induction of negative controls (cyclin inhibitors) by the binding of this hormone to its VDR receptor (Fleet, DeSmet, Johnson, & Li, 2012). In a study carried out by Rots, Iavarone, Bromleigh, and Freedman (1999), after 1,25(OH)<sub>2</sub>D addition in the myelomonoblastic leukaemic cell line U937, there was an increase in the expression of p21 Waf1/Cip1 (whose transcription is directly regulated by the presence of this) and p27Kip1, suggesting that the control of cellular differentiation mediated by this hormone was related to the ability to raise negative controller concentrations and interrupt the cell cycle in G1 phase.

### *Insulin-like growth factor*

1,25(OH)<sub>2</sub>D may also indirectly influence cell growth by interfering in growth factors action which stimulates proliferation or increases production of promoting cell differentiation factors. In MCF-7 human breast tumour cell lines, cell growth stimulated by Insulin-Like Growth Factor (IGF) was inhibited by vitamin D analogues and its effect was associated with increased release of IGFBP3 (IGF-binding protein 3) into the medium (Colston, Perks, Xie, & Holly, 1998). It is known that IGFBP3 limits the anti-apoptotic action of IGF1 and IGF2 by binding to them and limiting their ability to interact with cell surface receptors.

### *Growth transforming factor (TGFβ-transforming growth factor β)*

1,25(OH)<sub>2</sub>D also plays an anti-proliferative role in promoting increased expression of TGFβ2, which is considered an anti-proliferative factor for healthy epithelial cells and in the early stages of carcinogenesis. Generally, in cells of epithelial origin, the members of the TGF-β superfamily act as tumour suppressors, by preventing the progression of the cell cycle. Studies with different breast tumour cell lines (Lee et al., 2006; Swami, Raghavachari, Muller, Bao, & Feldman, 2003) and prostate (Peehl et al., 2004) have shown that 1,25(OH)<sub>2</sub>D and its analogues have promoted increased expression of TGFβ2. In addition, it has been demonstrated in MCF-7 breast cancer cells that calcitriol induces the expression of TGF-β1 and TGF-β receptors (Yang, Yang, Venkateswarlu, Ko, & Brattain, 2001).

### *Apoptosis*

*In vitro* studies have shown that 1,25(OH)<sub>2</sub>D can influence cell apoptosis by different mechanisms. In HGC-27 cell lines of gastric cancer,

1,25(OH)<sub>2</sub>D promoted apoptosis through increased PTEN gene expression (Pan et al., 2010), a tumour suppressor gene. In LNCaP prostate adenocarcinoma cell lines, 1,25(OH)<sub>2</sub>D promoted apoptosis, probably due to decreased expression of the anti-apoptotic proteins Bcl-2 and Bcl-XL1, which was observed in this study (Blutt, McDonnell, Polek, & Weigel, 2000). Other in vitro studies have demonstrated that 1,25(OH)<sub>2</sub>D treatment induces apoptosis by increasing the gene expression of pro-apoptotic proteins (Lin et al., 2002; Pálmer et al., 2003) or decreasing the expression of anti-apoptotic proteins (Kizildag, Ates, & Kizildag, 2010).

### Metastasis

Genetic mutations accumulated during tumour development result in dysregulation of important signal transduction pathways, such as the Wnt-β catenin signalling pathway, which is related to malignant transformation of tumours. In lineages of prostate, breast and colorectal cancer cells, 1,25(OH)<sub>2</sub>D treatment has promoted down-regulation of Wnt signalling pathway through VDR interaction with β-catenin (Kovalenko, Zhang, Cui, Clinton, & Fleet, 2010; Pendas-Franco, Aguilera, Pereira, González-Sancho, & Munoz, 2008; Swami et al., 2003). This appears to be related to decreased tumour metastasis. In addition, it has been shown that treatment with 1,25(OH)<sub>2</sub>D in colorectal cancer cell lines promotes up-regulation of DKK-1, the Wnt antagonist (Aguilera et al., 2007).

## 5.2.2 | Angiogenesis

Angiogenesis is necessary for metastasis development. This process depends on several pro-angiogenic factors. 1,25(OH)<sub>2</sub>D appears to decrease the expression of pro-angiogenic factors and increase the expression of anti-angiogenic proteins. It has already been demonstrated in tumours derived from mouse endothelial cells that treatment with 1,25(OH)<sub>2</sub>D decreased angiopoietin-2, an angiogenic signalling molecule, and this contributed to the proliferation inhibition of these tumours (Bernardi, Johnson, Modzelewski, & Trump, 2002). In lineages of SW480-ADH colon cancer cells, 1,25(OH)<sub>2</sub>D reduced the expression of the vascular endothelial growth factor (VEGF), considered the main pro-angiogenic factor and also increased the expression of thrombospondin-1, an anti-angiogenic protein (Fernandez-Garcia et al., 2005).

As in IBD, it is unclear whether low vitamin D status in dogs with neoplasia is the cause or only a consequence of the disease, much less what mechanisms are involved in that process. All studies that investigated the relationship between vitamin D and cancer incidence had one or more limitations, such as non-standardization of diets, no analysis of the vitamin D content of the diets, a small number of animals and impossibility of breed standardization. In addition, all studies evaluated the concentrations of 25(OH)D only at the time of diagnosis, and there were no evaluations prior to the development of the neoplasia.

Vitamin D has also been shown to be an effective agent in suppressing the development and progression of tumours in mice used

as an experimental model of colorectal cancer and ovarian cancer when used alone (Ness, Miller, & Wei, 2015). These data could reinforce the hypothesis that low vitamin D status may contribute to the development of various types of cancer, but this has yet to be proven. Even if this was true, it remains to be shown which mechanisms are involved in decreasing concentrations of 25(OH)D that could contribute to tumour development. This lack of information exists because most of the studies that evaluated the vitamin D status in dogs with cancer did not perform laboratory analysis of vitamin D levels in the diet of animals that participated in the experiments. Therefore, it is not known whether lower vitamin D concentrations were simply caused by a lower dietary intake or if there is any other mechanism involved.

Weidner et al. (2017) observed that dogs with lymphoma ( $n = 27$ ), osteosarcoma ( $n = 21$ ) and mastocytoma ( $n = 21$ ) had lower serum 25(OH)D concentrations compared to a control group of healthy dogs. In this study, it was possible to collect samples of the foods consumed by the majority of dogs that participated in the experiment to analyse vitamin D levels. There was no correlation between dietary intake of vitamin D and serum 25(OH)D concentrations. The relationship between cancer and a change in vitamin D metabolism was suggested because in cancer patients, plasma 25(OH)D concentrations increased as ionized calcium increased, whereas in healthy dogs, plasma 25(OH)D concentrations decreased with the increase of ionized calcium concentrations. Only dogs with low concentrations of ionized calcium had a significant decrease in plasma 25(OH)D concentrations when compared to healthy dogs. However, it was also not possible to determine whether this change in metabolism, observed in dogs with cancer, is associated with the development of the neoplasia, or if it is a consequence of the disease.

In humans, an association between polymorphism of vitamin D receptor gene and cancer has been demonstrated (Köstner et al., 2009) which might also occur in pets, but this has not yet been studied in dogs and cats.

## 5.3 | Vitamin D and congestive heart failure

Evidence suggests an involvement of vitamin D in the pathophysiology of heart disease (Witham, 2011). It is known that 1,25(OH)<sub>2</sub>D regulates cardiac remodelling by exerting an anti-hypertrophic effect on cardiomyocytes and modulates myocardial extracellular matrix turnover (Weber, Weglicki, & Simpson, 2008). Studies in experimental animals have shown that 1,25(OH)<sub>2</sub>D promotes cardiac contractility by binding to VDR in cardiomyocytes, exerting effects on intracellular calcium (Green, Robinson, Wilson, Simpson, & Westfall, 2006).

In humans, epidemiological studies have shown that lower vitamin D concentrations are a risk factor in patients with cardiovascular disease and is associated with congestive heart failure (CHF), left ventricular hypertrophy, chronic vascular inflammation, coronary disease and arterial hypertension (Lee, O'Keefe, Bell, Hensrud, & Holick, 2008). Low concentrations of 25(OH)D are associated with

cardiac remodelling and dysfunction, severe symptoms of heart failure and unfavourable prognosis (Fall et al., 2012). This association was even more evident in a study carried out with genetically modified mice that did not express VDR, which developed classic signs of CHF, such as cardiac hypertrophy, arterial hypertension and increased concentrations of atrial natriuretic peptide (Xiang et al., 2005).

Although no studies regarding vitamin D and cardiovascular disease in cats were found in the literature, two studies evaluated serum 25(OH)D in dogs with CHF. In the first study conducted by Kraus et al. (2014), significantly lower serum 25(OH)D concentrations in dogs with CHF compared to healthy dogs were observed. These same authors, in a prospective study, demonstrated an association between low serum 25(OH)D concentrations and worse clinical outcomes, as dogs with CHF and lower levels of 25(OH)D presented a significantly shorter period before developing clinical signs of the disease. Despite no difference was found in the daily intake of vitamin D between the groups, a laboratory evaluation of the vitamin D content in the foods was not performed and daily vitamin D intake was only estimated using the information given by the manufacturer or presented on labels, and information regarding the amount of food consumed and others diet history information provided by the owners through a questionnaire applied to them. In a second study, an association between vitamin D and degree of cardiac remodelling was observed, in which concentrations of 25(OH)D were significantly lower in the B2 and C/D stages compared to the B1 stage of CHF (Osuga et al., 2015). In this study, a calculation was not performed to estimate the daily intake of vitamin D by the animals, and there was no laboratory evaluation of the foods consumed.

Regarding VDR, a recent study conducted by Cartwright et al. (2018) evaluated its expression in different tissues of dogs. Organs that had a higher overall score of expression in immunohistochemistry were duodenum, ileum, kidney and skin, confirming previous findings by Palm, Hartmann, and Weber (2010). This was expected by the authors, as these tissues participate in the calcium homeostasis. Heart tissue, however, was labelled weakly for VDR expression, although it is uncertain what this information means taking into consideration the role of vitamin D in the development of heart diseases in dogs.

In humans, vitamin D supplementation in CHF patients has been shown to reduce inflammation (Schleithoff et al., 2006) and mortality (Gotsman et al., 2012), increase ejection fraction and decrease systolic blood pressure (Dalbeni et al., 2014). A research group, however, did not observe the effect of supplementation of vitamin D in the outcome of patients with advanced heart failure (Zittermann et al., 2017). This group performed the EVITA trial, which aimed to evaluate the effects of vitamin D on different parameters of heart failure in humans. A total of 400 patients received either 4,000 IU of vitamin D<sub>3</sub> or placebo for 36 months, and no difference was observed in anaemia prevalence or in cardiac function when compared to the placebo group (Zittermann et al., 2019).

Given the lack of vitamin D status information in dogs with CHF, it is not possible to determine whether low serum 25(OH)D

concentrations influenced the development of heart disease or occurred as a consequence of the disease. In addition, it remains to be studied if dogs and cats with CHF would benefit from vitamin D supplementation, as it is still controversial in studies in humans.

## 5.4 | Vitamin D as a predictor of mortality

Low concentrations of 25(OH)D are associated with increased overall mortality in the human population (Melamed, Michos, Post, & Astor, 2008), time of hospitalization (De Pascale et al., 2016; Higgins et al., 2012; Matthews, Ahmed, Wilson, Griggs, & Danner, 2012) and disease severity (Anwar et al., 2017). Meta-analysis studies have shown that serum concentrations of 25(OH)D may be independent predictors of survival in people with a wide variety of diseases (Schöttker et al., 2014), and the prevalence of lower vitamin D concentrations in critically ill people is about 82% (Melamed et al., 2008). However, the mortality of human patients with advanced heart failure supplemented with vitamin D for 3 years did not differ from that of patients that received placebo for the same period of time (Zittermann et al., 2017).

In veterinary medicine, there are studies that measured serum 25(OH)D and other clinical, biochemical and haematological parameters in hospitalized dogs and cats. In a study involving 99 hospitalized cats, although 25(OH)D concentrations were higher in cats that were alive after 30 days when compared to cats that died, it was not considered a predictor of mortality as there was not a linear regression (Titmarsh, Kilpatrick, et al., 2015). Kim et al. (2017) observed that dogs that survived to acute pancreatitis had concentrations of 25(OH)D higher than concentrations observed in dogs that died due to this disease before the beginning of treatment. Jaffey, Backus, McDaniel, and DeClue (2018) found that dogs critically ill or with sepsis had lower serum 25(OH)D concentrations compared to healthy control dogs. Besides, serum 25(OH)D was considered a good predictor of survival for dogs in the intensive care unit and 30 days after discharge and it presented a correlation with illness severity.

The increased risk of mortality in critically ill patients with hypovitaminosis D may be associated with the various pleiotropic effects of vitamin D. The authors associated these effects to the pleiotropic effects of vitamin D in immunity, endothelial and mucosa functions which are also found in humans. They believe that these effects are caused by the increase in the innate immune response caused by the vitamin D by enhancing phagocytosis and the induction of antimicrobial peptides, cathelicidin and beta-defensins (Baeke, Gysemans, et al., 2010; Baeke, Takiishi, Korf, Gysemans, & Mathieu, 2010; Jeng et al., 2009; Leaf, Raed, Donnino, Ginde, & Waikar, 2014; Liu et al., 2006). The authors also considered that the effects of calcitriol, which down-regulates the leucocyte production of pro-inflammatory cytokines such as IL-1, IL-2, TNF-alpha, and IL-6 and also increases the production of the anti-inflammatory cytokine IL-10, as it does in humans (Grubczak et al., 2015; Harishankar, Afsal, Banurekha, Meenakshi, & Selvaraj, 2014; Neve, Corrado, &

Cantatore, 2014; Villaggio, Soldano, & Cutolo, 2012) could also explain the correlation between the prognostic of ill/septicaemic dogs and vitamin D levels. Considering these facts, the authors hypothesized that the decreased circulating 25(OH)D reservoir needed for paracrine production of calcitriol is diminished in critically ill patients which results in compromised innate immune function and an exaggerated pro-inflammatory milieu.

However, with these studies alone, it was not possible to determine whether concentrations of 25(OH)D below reference levels were determinant for higher mortality, or whether this was only a result of greater severity of the disease. It remains to be seen whether vitamin D supplementation in hospitalized dogs and cats would reduce mortality. In humans, vitamin D supplementation is associated with a decrease in the total mortality rate of the population (Autier & Gandini, 2007).

## 5.5 | Vitamin D and immune function

It is known that vitamin D acts on the immune system, through different mechanisms. It is believed that the immunomodulatory role of vitamin D is triggered by its binding to the vitamin D receptor (VDR), which is expressed in several antigen-presenting cells such as monocytes, macrophages and dendritic cells (Baeke, Gysemans, et al., 2010; Baeke, Takiishi, et al., 2010; Hart, Gorman, & Finlay-Jones, 2011). As effects, it promotes an increase in innate immune response by increasing phagocytosis (Motlagh, Ahangaran, & Froushani, 2015) and induction of the synthesis of antimicrobial peptides such as cathelicidins and  $\beta$ -defensins (Liu et al., 2006). Furthermore, 1,25(OH)<sub>2</sub>D is known to play an autocrine immunoregulatory role in several immune cells, such as T lymphocytes, CD4+, CD8+ and antigen-presenting cells (Hewison, 2010). Besides, it also reduces the production of pro-inflammatory cytokines (e.g., Tumour necrosis factor- $\alpha$  and IL-6) while increases the production of IL-10, which has an anti-inflammatory potential (Grubczak et al., 2015; Harishankar et al., 2014; Neve et al., 2014; Villaggio et al., 2012). Vitamin D is capable of interfering in the TNF production without impacting phagocytosis in neutrophils and monocytes in dogs (Jaffey, Amorim, & DeClue, 2017).

Vitamin D promotes the transformation of CD4 T-helper (Th)1 cells to Th2 polarity in some autoimmune condition and promotes a state of immune "tolerance" (Baeke, Gysemans, et al., 2010; Baeke, Takiishi, et al., 2010) in humans. In dogs, a study evaluated vitamin D concentration in animals with immunomediated disease and hypothesized that serum 25(OH)D might reflect a predisposition to immune dysfunction (Grobman, Outi, Rindt, & Reinero, 2017). This information can correlate with the findings in humans, where possibly a low vitamin D concentration would lead to a "non-tolerance" state of immunity. Also regarding autoimmune diseases, a retrospective study evaluated if dogs with acute polyradiculoneuritis have lower serum 25(OH)D concentration compared to a control group of dogs with idiopathic epilepsy (Laws et al., 2018). The authors found that when affected by

acute canine polyradiculoneuritis, the dogs had significantly lower serum 25(OH)D concentrations compared to a control group with idiopathic epilepsy.

It also has been shown that vitamin D decreases Th1/Th17 CD4+ T cells and cytokines, while it increases regulatory T cells. Vitamin D also induces down-regulation of T cell-driven immunoglobulin G (IgG) production and inhibition of dendritic cell differentiation (Penna & Adorini, 2000). Aiming to determine the effects of vitamin D in critically ill dogs, Jaffey, Amorim, et al. (2018) evaluated *in vitro* the effect of calcitriol on cytokine production from whole blood collected from critically ill dogs. As a result, the calcitriol increased IL-10 production and decreased TNF- $\alpha$ , without altering the production of IL-6. These data suggest that calcitriol induces an anti-inflammatory phenotype in whole blood from critically ill dogs, when evaluated *in vitro*. These findings indicate that calcitriol may have a potential as an immunomodulatory therapy in dogs, although the authors believe that it is possible that calcitriol effects on immunologic milieu are dependent on the type and severity of the illness in dogs. In a similar study regarding vitamin D, its effect on indicators of the immune system was evaluated. Canine blood samples that were exposed to calcitriol, both primed with lipopolysaccharide (LPS) or phosphate-buffered saline (PBS), had a significant decrease in TNF production, and when LPS-primed samples were exposed to calcitriol, there was an increase in interleukin 10 production, without compromising neutrophil and monocyte viability (Jaffey et al., 2017). This suggests a potential anti-inflammatory effect on canine leucocytes.

## 5.6 | Vitamin D and infectious disease

Considering the effects of vitamin D over immunity it is expected that it is correlated to the susceptibility to infectious diseases. Concentrations of 25(OH)D have been measured in dogs and cats who presented some infectious disease. Low concentrations of 25(OH)D were found in dogs with neoplastic and non-neoplastic spirocercosis compared to healthy animals (Higgins et al., 2012). Rodriguez-Cortes et al. (2017) observed an association between lower vitamin D concentrations and the progression of visceral leishmaniasis in dogs. In a recent study, O'Brien, McMichael, and Le Boedec (2018) observed that dogs naturally affected by blastomycosis had lower circulating concentrations of 25(OH)D when compared to healthy dogs. These authors also observed an association between 25(OH)D concentrations and neutrophil count, partial carbon dioxide pressure, and the bone and cutaneous involvement of the illness. In cats affected by mycobacteriosis, concentrations of 25(OH)D were lower than those found in healthy animals (Lalor et al., 2012). Titmarsh, Lalor, et al. (2015) observed lower concentrations of 25(OH)D in cats infected with feline immunodeficiency virus than in healthy cats.

Concerning dogs with babesiosis, a study was developed evaluating proteomics, including the proteins which bind to vitamin D in the metabolism pathway. The authors found that in these dogs,

the vitamin D-binding protein is down-regulated. This protein function is to carry vitamin D and its plasma metabolites (Kuleš et al., 2014).

Regarding leishmaniosis, it is known that some protective responses are associated with the activation of specific cell-mediated immunity and a Th1-pro-inflammatory immune response (Rodríguez-cortes et al., 2017). It seems that Th17 cells act synergistically with the Th1 population to control the growth of the pathogen. When the animal has the active disease, it has been linked to a high antibody level and a progressive Th2-deactivating immune response in the presence of a strong inflammatory reaction (Rodríguez-Cortés et al., 2016). The authors believe that there are evidences suggesting that the innate immune system could have a relevant role by promoting the appropriate inflammatory response against the development of the disease (Bhattacharya et al., 2015). The authors found that dogs which had the disease had lower vitamin D levels than non-infected and asymptomatic animals. Besides, lower vitamin D concentrations was correlated with several parameters linked to leishmaniasis progression. On the other hand, no correlation has been found between vitamin D levels and the *Leishmania*-specific cellular immune response (Rodríguez-Cortés et al., 2017).

Considering that hypovitaminosis D is usually observed in humans with tuberculosis, Lalor et al. (2012) developed a study to verify whether spontaneous mycobacteria infections in other species were also associated with low vitamin D status. Lower serum 25(OH)D concentrations were indeed detected in cats with mycobacteriosis in comparison to healthy cats. That is an important finding, once there is already some evidence that vitamin D supplementation may increase the immunity to mycobacteria (Martineau et al., 2007). 1,25(OH)<sub>2</sub>D has shown an anti-mycobacterial activity in vitro (Liu et al., 2006) by the induction of reactive nitrogen and oxygen intermediates, suppression of matrix metalloproteinase enzymes (which is associated to the pathogenesis of pulmonary cavitation) (Anand & Selvaraj, 2009) and also by the induction of the antimicrobial peptide cathelicidin (Liu et al., 2006). These authors compared the 25(OH)D concentrations between healthy, with mycobacteria and systemically ill cats and notified that although healthy cats have higher concentrations of vitamin D, the other groups were similar, which may indicate that lower 25(OH)D concentrations are a non-specific feature of systemic illness in cats. On the other hand, the authors also believed that further studies are still needed; once appetite and food ingestion were no quantified and since systemic ill cats tend to have less appetite/lower food ingestion than mycobacterial infected cats.

Considering that hypovitaminosis D is associated with neoplasia and spirocercosis infection may also induce tumoral formation, Rosa et al. (2013) developed a study to compare vitamin D status in healthy, neoplastic and non-neoplastic dogs with spirocercosis. The authors found that 25(OH)D concentrations were significantly lower in neoplastic group in comparison to the others. The appetite was also evaluated by the owners using a score of normal/abnormal (which was decreased appetite or total anorexia), but neoplastic

and non-neoplastic spirocercosis dogs presented similar appetite scores. In this study, all animals received the same diet, although the exact amount of food intake could not be reported. Serum 25(OH)D concentrations were not significantly different between dogs with normal ( $p = .087$ ) and abnormal appetites within the neoplastic and non-neoplastic spirocercosis groups.

Regarding to fungal infections, a case-control study with humans discovered a genetic association between the Gc-2 allele of vitamin D-binding protein and a reduction in the susceptibility to blastomycosis. It was stated that the Gc-2 allele can increase antimicrobial activity of macrophages, and the authors believe that it is possible to mimic the mechanism by supplementing vitamin D (Sainsbury, Trajtman, Stalker, Embil, & Keynan, 2014). In another study, Liu et al. (2006) had also correlated the susceptibility to diseases to vitamin D status. In innate immune responses, the activation of toll-like receptors (TLR) is responsible for trigger direct antimicrobial activity against intracellular bacteria. The authors stated that TLR activation of macrophages up-regulates the expression of the vitamin D receptor, which leads to the induction of the antimicrobial peptide cathelicidin. It has also been noticed that individuals African Americans, which are more susceptible to tuberculosis, had low 25(OH)D and were inefficient to produce cathelicidin. As a conclusion to these findings, the authors stated that there is a link between TLR and vitamin D-mediated immunity and suggests that the differences in ability of human population to produce vitamin D may contribute to susceptibility to microbial infection.

## 5.7 | Chronic kidney disease

There are several studies that evaluated vitamin D metabolism in patients with Chronic kidney disease (CKD), since its active metabolite (1,25(OH)<sub>2</sub>D) is produced in the renal tubules through the enzyme 1 $\alpha$ -hydroxylase (de Brito Galvao, Nagode, Schenck, & Chew, 2013). Studies have shown that dogs with CKD have serum and plasma concentrations of the 25(OH)D and 1,25(OH)<sub>2</sub>D metabolites lower than the concentrations found in healthy dogs (Cortadellas et al., 2010; Galler et al., 2012; Gerber et al., 2003, 2004; Parker, Harjes, et al., 2017).

In patients with CKD, several factors may influence vitamin D status in the body, such as decreased activity of the 1 $\alpha$ -hydroxylase enzyme (Parker, Harjes, et al., 2017), reduced hepatic conversion of cholecalciferol to 25(OH)D (Michaud et al., 2010) and decreased dietary intake of vitamin D. Furthermore, it is known that 25(OH)D bound to DBP is submitted to the glomerular filtration process before being converted to 1,25(OH)<sub>2</sub>D in the renal tubules, and its reabsorption occurs via endocytosis mediated by megalin receptor. As a consequence of CKD, there is a decline in this endocytic activity, which causes loss of the 25(OH)D-DBP complex in urine (Li, 2013). This loss in urine can also occur as a consequence of proteinuria (Pérez-Gómez, Ortiz-Ardúan, & Lorenzo-Sellares, 2013). Another important factor that may influence vitamin D status of patients with CKD is FGF-23, a hormone produced by bone tissue that

stimulates renal phosphate excretion and promotes suppression of 1,25(OH)<sub>2</sub>D synthesis by inhibiting the 1 $\alpha$ -hydroxylase enzyme and stimulating 24-hydroxylase (Shimada et al., 2004).

One of the consequences of CKD is the development of secondary renal hyperparathyroidism (SRH). As one of the treatments for SRH, it is recommended the use of calcitriol (Nagode, Chew, & Podell, 1996). However, Hostutler et al. (2006) found no difference in serum PTH concentrations after 14 days of oral calcitriol treatment at the dosages of 2.5 ng/kg every 24 hr, or 8.75 ng/kg every 84 hr in cats with CKD and in healthy cats, as no difference was found in serum ionized calcium concentrations. Further studies are needed to evaluate the effects of higher calcitriol dosages on the reduction of serum PTH in patients with CKD, as well as to evaluate whether in dogs and cats affected by this disease calcitriol or analogues could promote the effects observed in humans, such as antiproteinuric effect (Agarwal et al., 2005) and the increase in life expectancy in patients with CKD (Shoben, Rudser, De Boer, Young, & Kestenbaum, 2008).

Besides the decrease in serum PTH and proteinuria, there is evidence that calcitriol and its analogues act on regulation of: renin-angiotensin-aldosterone system (Yuan et al., 2007), NF-Kappa B protein complex (Sun et al., 2006), Wnt/ $\beta$ -catenin pathway (Shah et al., 2006) and the slit diaphragm proteins (Zhang et al., 2009), which are involved in the process of injury and renal damage. This could also be related to the increased survival time CKD human patients undergoing administration of calcitriol analogues. However, it is noteworthy that care should be taken regarding the use of calcitriol, because an overdose of this metabolite can cause hypercalcemia, which may result in acute renal tubular necrosis and calcinosis, such as nephrocalcinosis (Peterson & Fluegeman, 2013). This is extremely damaging, especially for animals that already have CKD, so the recommendation should ensure an adequate but not excessive supply.

## 6 | OTHER PUBLICATIONS

Based on the association between vitamin D and inflammation in human medicine, a correlation between circulating concentrations of 25(OH)D and the severity of atopic dermatitis signs in children has been demonstrated (Peroni, Piacentini, Cametti, Chinellato, & Boner, 2011). In veterinary medicine, a recent study investigated the effects of systemic administration of cholecalciferol in dogs with atopic dermatitis (Klinger et al., 2018). In this study, it was observed that dogs in the treatment group had lower lesion and pruritus scores when compared to those of the placebo group. It was also observed that the increase in serum concentrations of 25(OH)D had a correlation with decreased pruritus.

## 7 | CONCLUSIONS

Dogs and cats have a different vitamin D metabolism; therefore, they are dependent on the dietary intake of vitamin D. Low 25(OH)

D concentrations are associated with several diseases unrelated to bone metabolism, most studies were performed in humans or translational models for humans (i.e., rodents), and not much research specifically on cats and dogs, but some interesting findings point out possible similarities. Based on the data available in the literature until now, it is not possible to conclude whether lower vitamin D concentrations are influencing the development of these diseases, or are only a consequence of them. Further studies are needed to determine dietary vitamin D requirements for adult animals, as well as determining the ideal circulating 25(OH)D concentrations. Then, in a second step, the evaluation of the potential benefits of vitamin D supplementation on outcome in diseases.

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## CONFLICTS OF INTEREST

All authors confirmed that there are no conflicts of interest.

## ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a review article with no original research data.

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