Research, Society and Development, v. 10, n. 3, e57310313607, 2021
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v10i3.13607

Clinical and hematological evaluation in dogs with myoclonus derived from canine distemper supplemented with vitamin D_3

Avaliação clínica e hematológica em cães portadores de mioclonia decorrente de cinomose canina suplementados com vitamina D_3

Evaluacione clínica y hematológica en perros con mioclonías por moquillo canino suplementados con vitamina D_3

Received: 03/06/2021 | Reviewed: 03/12/2021 | Accept: 03/19/2021 | Published: 03/27/2021

Sarah Carvalho Oliveira Lima Dóro
ORCID: https://orcid.org/ orcid.org/0000-0001-5246-557X
Universidade Federal de Jataí, Brazil
E-mail: sari.rv@hotmail.com
Andréia Vitor Couto do Amaral
ORCID: https://orcid.org/ 0000-0001-6406-2372
Universidade Federal de Jataí, Brazil
E-mail: andreiavcvet@ufg.br

Abstract
In dogs, the synthesis of vitamin D in the skin is considered inefficient, making dietary supplementation the main source of this vitamin for these animals. In humans, there are established values for 25-hydroxyvitamin D (25(OH)D) deficiency, insufficiency and sufficiency levels, however in dogs, the serum concentrations of these values are not well established. The purposes of this study were to evaluate the 25(OH)D serum levels in dogs carrying myoclonus as sequelae of distemper, to evaluate the response to vitamin D levels on oral supplementation, to evaluate PTH, calcium, phosphorus, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood count and leukogram levels, in addition to conduct clinical observations of myoclonus. Venous blood samples were collected from nine dogs carrying myoclonus derived from distemper, however with no other clinical or laboratorial change, of varied breeds and same age group (1 - 8 years old). Screening laboratory tests were performed to attest to the health of the animals in a 30-day period and the collections were divided into three periods: days 0, 15 and 30. After the initiation of treatment, the animals underwent physical and laboratorial evaluations every 15 days, for 90 days, completing a total of 120 days. The dose used for oral supplementation of vitamin D_3 was 1000IU/kg administered every day, once a day, during the entire experimental period. For clinical evaluation, parameters of anatomical distribution, speed and rhythm, and distribution of myoclonic changes over time were observed. The laboratory results were subjected to analysis of variance and, when significant ($P<0.05$), submitted to regression analysis. Descriptive statistics were used to analyze clinical results. There was a significant difference in blood concentrations of 25(OH)D, PTH, calcium and phosphorus, however there was no significant effect of vitamin D on the other parameters evaluated. It was possible to conclude that the dose of vitamin D_3 used was sufficient to increase 25(OH)D serum levels in the blood, to levels of sufficiency, having influence on PTH, phosphorus and calcium levels, not changing the other hematological and clinical parameters evaluated. However, the dose and duration of the treatment used did not change the myoclonus derived from distemper in dogs.
Keywords: - 25-hydroxyvitamin D; Cholecalciferol; Parathyroid hormone.

Resumo
Em cães, a síntese de vitamina D na pele é considerada ineficiente, fazendo da suplementação dietética a principal fonte desta vitamina para esses animais. Em humanos, há valores estabelecidos para níveis de deficiência, insuficiência e suficiência de 25-hidroxivitamina D (25(OH)D), porém em cães, as concentrações séricas desses valores não são bem estabelecidas. O presente estudo teve como objetivos avaliar os níveis séricos de 25(OH)D em cães portadores de mioclonia como sequela da cinomose, avaliar a resposta aos níveis de vitamina D perante suplementação oral, avaliar os níveis de PTH, cálcio, fósforo, alanina aminotransferase (ALT), aspartato aminotransferase (AST), hemograma e leucograma, além de realizar observações clínicas das mioclonias. Foram coletadas amostras de sangue venoso de nove cães portadores de mioclonias em decorrência da cinomose, entretanto sem quaisquer outras alterações clínica ou laboratorial, de raças variadas e mesma faixa etária (1 - 8 anos). Exames laboratoriais de triagem foram realizados para atestar a saúde dos animais em um período de 30 dias e as coletas foram divididas em três períodos: dias 0, 15 e 30. Após o início do tratamento, os animais passaram por avaliações físicas e laboratoriais a cada 15 dias, durante 90 dias, completando um total de 120 dias. A dose utilizada para

suplementação oral de vitamina D_3 foi de 1000UI/kg administrada todos os dias, uma vez ao dia, durante todo o período experimental. Para a avaliação clínica, foram observados os parâmetros de distribuição anatômica, velocidade e ritmo e distribuição no tempo das alterações mioclônicas. Os resultados laboratoriais foram submetidos à análise de variância e, quando significativos (P<0,05), foram submetidos à análise de regressão. Para a análise dos resultados clínicos foi utilizada estatística descritiva. Houve diferença significativa nas concentrações sanguíneas de 25(OH)D, PTH, cálcio e fósforo, mas não houve efeito significativo da vitamina D nos demais parâmetros avaliados. Foi possível concluir que a dose utilizada de vitamina D_3 foi suficiente para aumentar os níveis séricos de 25(OH)D no sangue, a níveis de suficiência, tendo influência nos níveis ne PTH, fósforo e cálcio, sem alterar os demais parâmetros hematológicos e clínicos avaliados. Entretanto, a dose e a duração do tratamento utilizadas não alteraram a mioclonia decorrente da cinomose em cães.
Palavras-chave: - 25-hidroxivitamina D; Colecalciferol; Paratormonio.

Resumen
En los perros, la síntesis de vitamina D en la piel se considera ineficiente, por lo que la suplementación dietética es la principal fuente de esta vitamina para estos animales. En humanos, existen valores establecidos para los niveles de deficiencia, insuficiencia y suficiencia de 25-hidroxivitamina D (25 (OH) D), sin embargo, en perros, las concentraciones séricas de estos valores no están bien establecidas. El presente estudio tuvo como objetivo evaluar los niveles séricos de 25 (OH) D en perros con mioclonías como consecuencia del moquillo, evaluar la respuesta a los niveles de vitamina D con suplementación oral, evaluar los niveles de PTH, calcio, fósforo, alanina aminotransferasa (ALT), aspartato aminotransferasa (AST), hemograma y leucograma, además de realizar observaciones clínicas de mioclonías. Se recogieron muestras de sangre venosa de nueve perros con mioclonías por moquillo, pero sin otros cambios clínicos o de laboratorio, de diferentes razas y del mismo grupo de edad (1 - 8 años). Se realizaron pruebas de cribado de laboratorio para certificar la salud de los animales durante un período de 30 días y las recolecciones se dividieron en tres períodos: días 0, 15 y 30. Luego del inicio del tratamiento, los animales fueron sometidos a evaluaciones físicas y de laboratorio en cada 15 días, durante 90 días, completando un total de 120 días. La dosis utilizada para la suplementación oral de vitamina D_3 fue de 1000 UI / kg administrada todos los días, una vez al día, durante todo el período experimental. Para la evaluación clínica se observaron los parámetros de distribución anatómica, velocidad y ritmo y distribución temporal de los cambios mioclónicos. Los resultados de laboratorio se sometieron a análisis de varianza y, cuando eran significativos (P <0,05), se sometieron a análisis de regresión. Para el análisis de los resultados clínicos se utilizó estadística descriptiva. Hubo una diferencia significativa en las concentraciones sanguíneas de 25 (OH) D, PTH, calcio y fósforo, pero no hubo un efecto significativo de la vitamina D en los otros parámetros evaluados. Se pudo concluir que la dosis de vitamina D_3 empleada fue suficiente para incrementar los niveles séricos de 25 (OH) D en sangre, hasta niveles de suficiencia, influyendo en los niveles de PTH, fósforo y calcio, sin alterar las demás hematológicas y parámetros clínicos evaluados. Sin embargo, la dosis y la duración del tratamiento utilizado no alteraron el mioclono resultante del moquillo en los perros.
Palabras clave: - 25-hidroxivitamina D; Colecalciferol; Hormona paratiroidea.

1. Introduction

Vitamin D, currently defined as an important hormone, has been known for its role in osteomineral physiology, mainly for calcium and phosphorus regulation, promoting the absorption of those minerals by the intestinal mucosa (Davies *et al*., 2012). In human medicine, the search for the benefits of vitamin D for the extraskeletal health has become a frequent and expanding theme and its antineoplastic and immunomodulator effects have been studied (Weidner & Verbrugghe, 2017).

Although the development of research in this area for veterinary medicine is only beginning, there has been strong evidence that vitamin D has effects besides the osteomineral metabolism (Lips, 2006), as it is known that there is interaction of vitamin D receptors in more than 40 tissues acting in almost all body systems (Norman, 2012).

In humans, sun exposure remains the main source of vitamin D, both for most of the children and for adults (Holick, 2016; Holick, 2017). During sun exposure, 7-dehydrocholesterol, the immediate precursor in the biosynthetic pathway of cholesterol, absorbs ultraviolet B radiation (290 – 315nm), resulting in breakage of the link between carbon 9-carbon 10 to produce pre-vitamin D_3. Once formed, this unstable steroid suffers a rearrangement of its triene system to form the thermodynamically stable vitamin D_3 (cholecalciferol). Following, it is led to the liver, where it is converted into 25-hydroxyvitamin D. Then, this metabolite goes to the kidneys, where it is converted to the active form 1.25-di-dihydroxyvitamin D (Holick, 2007; Wacker & Holick, 2013).

In dogs, however, evidence suggests that the production of vitamin D in the skin, mediated by ultraviolet exposure, is essentially insignificant (Laws *et al*., 2018). High amounts of cholesterol have been found in lipid extracts of dog's skin, however no intermediate product of cholesterol synthesis, such as the precursor of vitamin D, 7-dehydrocholesterol (Wheatley & Sher, 1961). The absence of 7-dehydrocholesterol has formed the basis of the hypothesis that dogs have lost the ability to produce vitamin D, becoming dependent on its dietary intake (Weidner & Verbrugghe, 2017).

There is still no consensus on the acceptable blood concentrations of vitamin D for dogs, therefore, reference values remain undefined. However, citations for the optimal serum concentration of 25(OH)D in dogs are often found, such as the one from the Diagnostic Center for Population and Animal Health at the Michigan State University, which defined the interval between 43.6 - 169.2ng/mL (Nachreiner *et al*., 2014), and others suggesting the interval between 100 - 120ng/mL (Selting *et al*., 2016; Weidner & Verbrugghe, 2017).

Following medicine trends, there has also being interest in the extraskeletal effects of vitamin D and the role of the vitamin for health and disease conditions in companion dogs, since researchers have reported associations between low serum concentrations of 25(OH)D and canine mastocitoma tumors (Wakshlag *et al*., 2011), chronic kidney disease (Gerbe *et al*., 2003), congestive heart failure (Kraus *et al*., 2014) and inflammatory bowel disease (Gow *et al*., 2011).

The infection by the canine distemper virus may cause demyelination in different central nervous system regions (Orsini & Bondan, 2008), corresponding to the removal process of previously formed myelin sheaths (Santos, 2016). Demyelinating lesions are responsible for permanent sequelae, including myoclonus and the decreased quality of life of affected dogs. Thus, researchers have invested in alternative therapies with the purpose of optimizing the repair of these lesions in the central nervous systemet (Nunes, 2016; Santos, 2016).

A suggestive treatment for those lesions would be the use of vitamin D. Studies with the drug report the improvement of neurological lesions in autoimmune diseases and its probable benefit for the treatment of demyelination caused by the distemper virus in the neurological phase (Embry, Snowdon & Vieth, 2000).

According to Zhang and Ko (2009), vitamin D_3 stimulates the neutrophins responsible for regulating axonal growth and myelination, in addition to regulating neural death. Through that, the neurotrophic factors for the development and regeneration of the nervous system have an important role in pathological situations (Nunes, 2016).

The hypothesis of the study considers that daily supplementation with a dose of 1000IU/kg of vitamin D_3 (cholecalciferol) could decrease the myoclonus derived from distemper in dogs, evaluating the effective increase in vitamin D, PTH, total calcium, phosphorus, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea, blood count and leukogram levels and the clinical evaluation. Therefore, the purposes of this study were to identify the 25(OH)D levels in dogs carrying myoclonus derived from canine distemper and to evaluate the response to the levels on oral supplementation.

2. Methodology

The ethical approval for the study was obtained from the Ethics Committee on the Use of Animals/CEUA -Jataí (protocol 022/2017).

Nine adult male or female dogs carrying myoclonus derived from canine distemper, feeding normally with commercial balanced ration, examined at the Clínica São Francisco Vet Center in Rio Verde – GO, were selected to be included in the study. The informed consent for using the animals and the clinical blood samples for research purposes was obtained on admission for each dog selected. For selection, patients with a history of pharmacological vitamin D or calcium supplementation in the previous six months were excluded, as well as pregnant and breastfeeding patients, and those receiving

medications that affect vitamin D metabolism, or with a diagnosis of any disease that changes calcium or vitamin D metabolism, such as bone, parathyroid, renal and hepatic diseases.

Age, gender, breed and body weight were recorded for each dog. Blood samples were extracted from the jugular vein after antisepsis, in the period from day 0 to day 120, with an interval of 15 days between each collection, totaling nine samples. Analyses on days 0, 15 and 30 were performed to establish the health of the animals. The following clinical information was measured for each patient: parathormone, 25-hydroxyvitamin D, red blood cells, hematocrit, hemoglobin, MCV, MCH, MCHC, plasma proteins, platelets, total leukocytes, metamyelocytes, rod cells, eosinophil and basophil, lymphocytes, monocytes, ALT, creatinine, urea, AST, phosphorus and calcium.

For the measurement of paratormonium and 25-difroxyvitamin D, 2mL of blood were collected in a serum clot activator tube, according to the automated modular method Cobas 6000 (Roche). The hematological variables were measured in an Automated System SDH-3 VET. The biochemical parameters (ALT, AST, creatinine, urea, phosphorus and calcium) were collected in serum clot activator tubes and measured in a semiautomatic biochemical analyzer Spectrum - Celer.

Myoclonus was classified and measured, for each animal, according to anatomical distribution, frequency and rhythm intensity. According to distribution, it was defined as focal (1), segmental (2) or myoclonic twitch (3). According to the frequency of contractions, it was classified as intermittent (1), grouped and permanent (2) or continuous and permanent (3). According to speed or rhythm, it was classified as slow (0.05 - 1 contraction per second) (1), fast (2 - 8 contractions per second) (2), arrhythmic (3) or oscillatory (4) (Almeida, 1995). The evaluations of all animals were performed by the same professional, in the period from day 0 to day 120, with an interval of 15 days between each evaluation, totaling nine samplings.

The manipulation of vitamin was made in oily medium at the Laboratório de Manipulação Artesanal in Rio Verde - GO, determining 1000IU/drop in a total of 30mL per vial. The dose established per animal was 1 drop/kg per day, from day 31 to day 120 of the experiment.

For statistical analysis, the software Sisvar was used to obtain the analysis of variance (ANOVA) and the software Sigmaplot was used for regression graphs.

3. Results and Discussion

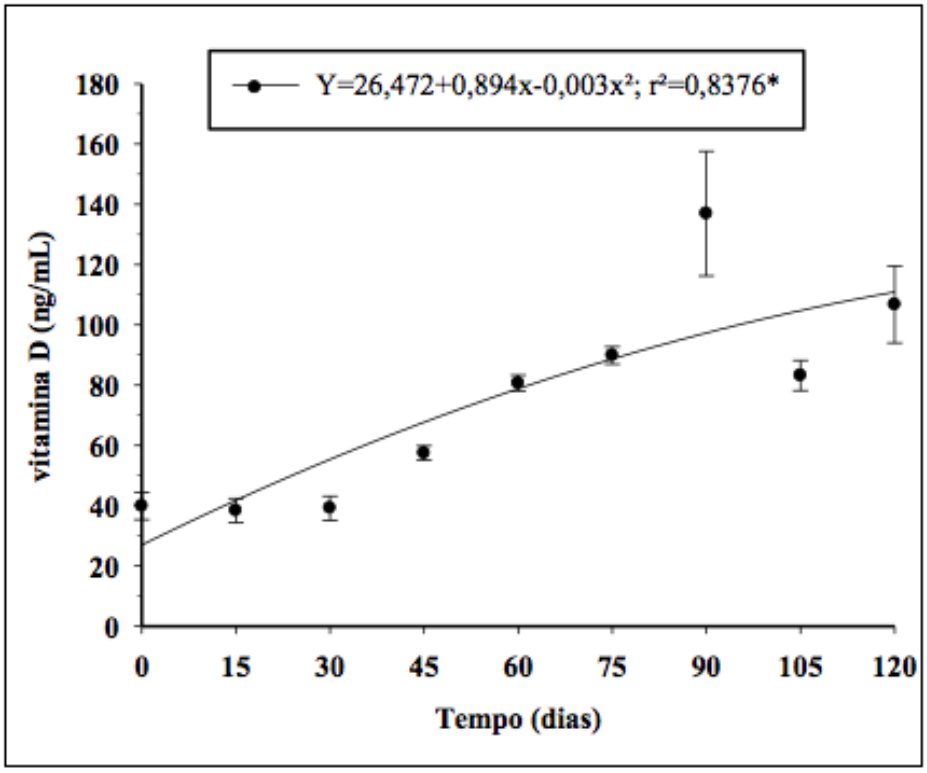
Nine dogs fulfilled the inclusion criteria, but only five were selected for statistical analysis by homogeneity based on their standard deviation.

According to the F Test, it was possible to observe that vitamin D_3 did not influence the ALP, hemoglobin, AST, ALT, urea, creatinine and hematocrit variables.

However, there was a difference in the supplementation of vitamin D_3 and in the values of vitamin D, calcium, phosphorus and parathormone found.

It was observed that the results of the 25(OH)D serum concentration on treatment days 15, 60 and 75, by quadratic regression, were closer to the overall mean of the group. Thus, it was possible to conclude that all dogs had a response similar to the treatment defined regarding the observation of this variable. It was possible to observe that the average concentrations of 25(OH)D obtained on days 0, 15 and 30 were between 20 and 40ng/mL. On days 60, 75 and 105, the values were close to 80 to 100ng/mL. On day 90, the average concentrations of 25(OH)D were between 140 and 160ng/mL and on day 120 between 100 and 120ng/mL (Figure 1).

Figure 1. Analysis of vitamin D concentration levels (ng/mL) obtained over time in dogs supplemented with vitamin D_3.



Y - variable evaluated
ng/mL – nanogram/per milliliter

[Y=26.472+0.894x-0.003x²; r²=0.8376*]
[vitamin D (ng/mL)]
[Time (days)]
Source: Authors.

The increased 25(OH)D serum concentration observed from the oral administration of vitamin D_3 occurred from day 45 to day 120. This result indicates that the dose used was sufficient to increase the serum concentration of this variable in dogs to significant levels. These results corroborate with Young and Backus (2017) who, using a dose of vitamin D_3 of 2.3g/kg of weight, observed significantly increased 25(OH)D serum levels in the second week of supplementation, reaching an average concentration of vitamin D of 112ng/mL.

The only research to address the concentration in dogs, using the chemiluminescence assay to measure the serum 25(OH)D, suggested as sufficiency 25(OH)D levels >100ng/mL, insufficiency between 25 and 100ng/mL and deficiency in concentrations <25ng/mL (Selting *et al*., 2016). Considering this classification, it was observed that in the period from day 0 to day 30, in which the animals were not supplemented, the 25(OH)D serum levels found were considered insufficient. At days 90 and 120, 25(OH)D serum concentrations with adequate sufficiency level were observed. These results imply that more research should be performed to establish hematological sufficiency, deficiency and insufficiency parameters in dogs and to establish a safe vitamin D_3 dose for daily supplementation, at health and disease conditions.

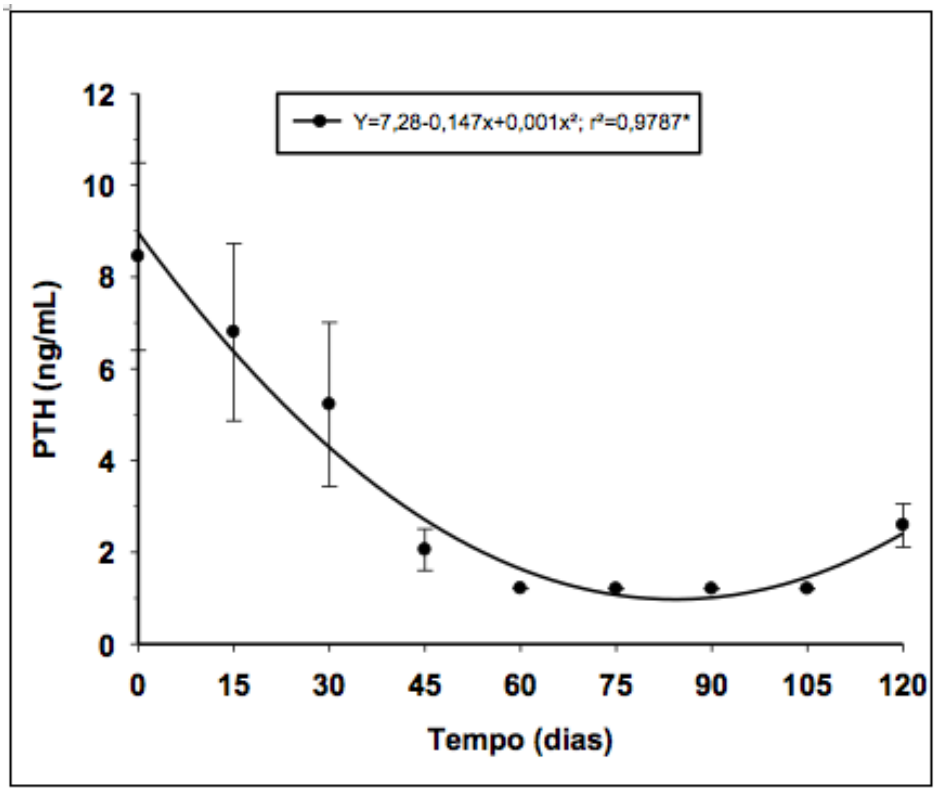
The 25(OH)D serum concentrations, found before the beginning of supplementation, are consistent with the findings by Young & Backus (2016), where 71.7% of the apparently healthy dogs evaluated had 25(OH)D serum concentration below 100ng/mL and consistent with the findings by Sharp *et al*., (2015) who, collecting samples from 320 dogs, observed that many apparently healthy dogs are insufficient in vitamin D while some others are deficient.

The reason for 25(OH)D being below the levels considered optimal for the species has not been defined yet, however it may be connected to deficiency in the synthesis of vitamin D by sun exposure, its dietary insufficiency and the fact that it is not supplemented.

Although the initial results were consistent, after the initiation of treatment different results were observed when compared to Young & Backus (2016), who, using a dose of 2.3g/kg of vitamin D_3 did not observe an increase in the concentration levels reported considered as sufficiency. It should be emphasized that the methods and tests to measure the 25(OH)D serum concentration are not standardized, which may result in different interpretations of results.

Regarding the parathormone, more consistent results were observed in the application of quadratic regression (Y=7.28-0.147X+0.001x2; r2=0.9787*), in which most of the values remained close to the overall mean of the group (Figure 2), confirming that the dogs responded similarly to the treatment proposed.

Figure 2. Graph of PHT analysis (ng/mL) showing concentration against time (days), evaluating PHT concentration over the period of vitamin D_3 administration in dogs carrying canine distemper myoclonus.



Y - variable evaluated
ng/mL – nanogram/per milliliter

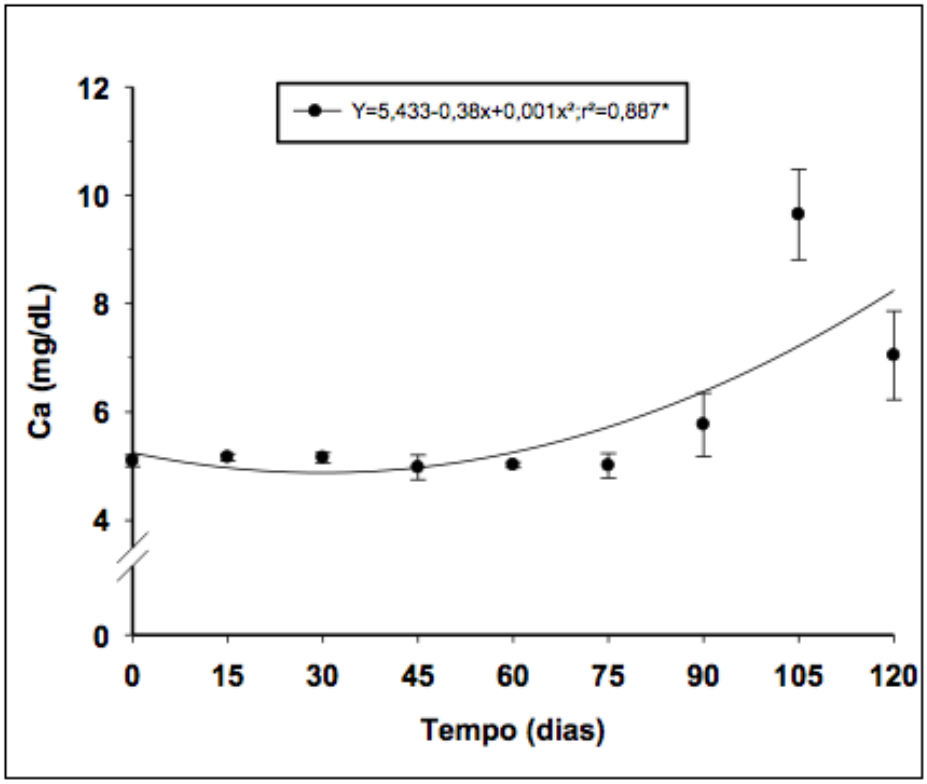
[Y=7.28-0.147x+0.001x²; r²=0.9787*]
[PTH (ng/mL)]
[Time (days)]
Source: Authors.

It was possible to observe that the serum levels of PTH decreased with increased concentrations of 25(OH)D. Observing the graphs from day 45, when the blood concentration of 25(OH)D began to increase, PTH levels began to decline and remained within the mean between 0 - 2ng/mL not seeming to stabilize and showing an increase in day 120 (2 - 4ng/mL). The same results were obtained by Selting *et al*., (2016), in which PTH continued to decrease with the increased concentrations of 25(OH)D.

In cases of vitamin D deficiency, there is a compensatory increase in PTH secretion that will stimulate the kidneys to produce the $1.25OH_2D_3$ (Marques et al., 2010). In contrast, with the increased vitamin D concentration, PTH levels decrease, therefore corresponding to the results obtained through PTH analysis.

In the calcium analysis, it was possible to observe, by quadratic regression, that means on days 0, 15, 30, 45 and 60 remained closer to the overall mean (Figure 3).

Figure 3. Analysis of calcium concentration (mg/dL) results over time (days), evaluating serum levels in a 120-day period in dogs with myoclonus.



Y - variable evaluated
mg/DL – milligrams per deciliter

[$Y=5.433-0.38x+0.001x^2$; $r^2=0.887$*]
[Ca (mg/dL)]
[Time (days)]
Source: Authors.

It was also observed that calcium serum levels increased during treatment. This result is explained as PTH, together with activated vitamin D, stimulates bone resorption by osteoclasts, thus increasing calcium serum concentrations (Barral *et al*., 2007). In the kidneys, vitamin D acts on the distal tubules promoting calcium reabsorption and, in the intestine, it acts on the endothelial cells stimulating the active calcium absorption in the duodenum and the passive absorption in the jejunum (Castro, 2011). This could explain the increased calcium serum levels observed in this study.

Between days 0 and 90, the mean of the total calcium serum concentration remained between 4.0 – 6.0mg/dL, increasing on day 105 (8.0 – 10.0mg/dL). According to Schenck (2008), the reference value of total calcium for dogs is 9.0 – 11.5mg/dL and of ionizable calcium is 5.0 - 6.0mg/dL. Therefore, it could be concluded that the mean obtained on day 105 was the closest to the optimal levels of total calcium.

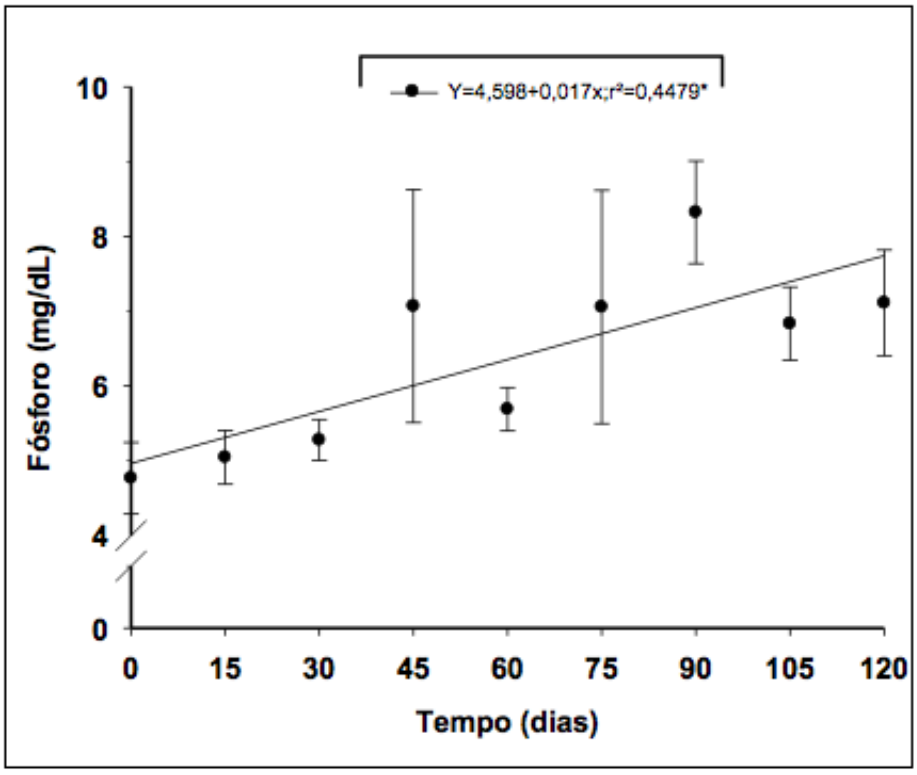
Regarding the phosphorus serum levels found in this study with application of simple linear regression, it was observed that both means were not close to the overall mean. When analyzing the graph of phosphorus concentration over time, it can be concluded that the serum levels increased linearly with supplementation of vitamin D_3 (Fig. 4). This linear increase is explained by the greater absorption of this ion in the intestine, stimulated by calcitriol (Abrita, 2015).

The role of vitamin D in maintaining the balance between calcium and phosphorus is already well established. In this study, it was observed that the 25(OH)D influenced the serum concentration of calcium and phosphorus. In contrast, there are studies that did not obtain the same observations. Fonseca (2017), for example, did not observe the direct correlation between the serum concentrations of total calcium and phosphorus and the levels of 25(OH)D. Similar results were also noted by Selting *et al*., (2016), who did not observe correlation.

When observing the results obtained in the clinical analysis, it was possible to confirm that, regardless of the time of use of vitamin D_3, the animals visually evaluated as to the anatomical distribution obtained mean, median and mode 2, most of them presenting segmental myoclonus, i.e., the myoclonic twitch affected two or more muscle groups in a specific body segment (Table 1).

Analyzing speed and rhythm results, it could be observed that 60% of the animals evaluated had slow rhythmic myoclonus from 0.5 to 1 contraction/second during the entire experimental period (Table 2). The rhythmic myoclonus has a specific, regular and uniform rhythm.

Figure 4. Analysis of phosphorus concentration levels (mg/dL) over time (days) in dogs carrying canine distemper myoclonus.



Y – variable evaluated
mg/DL – milligrams per deciliter

[Y=4.598+0.017x; r²=0.4479*]
[Phosphorus (mg/dL)]
[Time (days)]
Source: Authors.

Table 1. Descriptive statistics using the data of the five animals evaluated on Anatomical Distribution (AD), Speed and Rhythm (SR) and Distribution over Time (DT) for dogs evaluated with myoclonus in the 0 to 120-day period, before and after vitamin D_3 supplementation.

	Mean	Median	Mode	Standard Deviation	Sample Variance
AD	2	2	2	0.639602149	0.409090909
SR	1.4	1	1	0.495433694	0.245454545
DT	3	3	3	0	0

Source: Authors.

Table 2. Results obtained from myoclonus speed and rhythm of the animals selected for analysis, before and after daily vitamin D_3 supplementation for 90 days. Presentation of the results obtained for each evaluation period.

	ANIMALS				
DAY	**1**	**2**	**3**	**4**	**5**
0	1	1	2	1	2
15	1	1	2	1	2
30	1	1	2	1	2
45	1	1	2	1	2
60	1	1	2	1	2
75	1	1	2	1	2
90	1	1	2	1	2
105	1	1	2	1	2
120	1	1	2	1	2

1- Slow rhythmic myoclonus (0.5 to 1 contraction per second)
2- Fast Rhythmic Myoclonus (2 to 8 contraction per second)
Source: Authors.

For distribution over time, the mean, median and mode had value 3, and the animals visually evaluated were classified with continuous and permanent myoclonic twitches, happening continuously, periodically and for extended periods. Regarding the evaluation of the anatomical distribution in the speed and rhythm and the distribution of myoclonus over time, no differences were observed between the experimental periods for each animal evaluated (Table 3).

The results obtained from the clinical evaluations showed that there was no influence of vitamin D_3 on the evaluated parameters of speed and rhythm and distribution over time, that is, the animals did not show significant visual improvement to the treatment proposed. It is believed that this result was influenced by the experimental period, since the animals only had sufficiency levels as of the evaluation on day 90. We can suggest that the time for vitamin D_3 levels to present satisfactory concentrations for the treatment of myoclonic lesions was not sufficient.

Table 3. Results obtained from clinical evaluation of the five dogs evaluated during the 120-day period, considering vitamin D concentrations and the results in clinical analysis before and after oral supplementation with vitamin D_3.

ANIMAL	DAY	VITAMIN D (ng/mL)	AD	SR	DT
1	0	55.60	3	1	3
1	15	41.62	3	1	3
1	30	40.87	3	1	3
1	45	68.81	3	1	3
1	60	78.96	3	1	3
1	75	100.00	3	1	3
1	90	90.08	3	1	3
1	105	86.58	3	1	3
1	120	104.89	3	1	3
2	0	48.40	2	1	3
2	15	49.30	2	1	3
2	30	49.10	2	1	3
2	45	51.92	2	1	3
2	60	70.78	2	1	3
2	75	79.10	2	1	3
2	90	92.52	2	1	3
2	105	83.02	2	1	3
2	120	126.30	2	1	3
3	0	30.20	1	2	3
3	15	30.10	1	2	3
3	30	30.10	1	2	3
3	45	59.15	1	2	3
3	60	85.81	1	2	3
3	75	91.93	1	2	3
3	90	88.60	1	2	3
3	105	77.15	1	2	3
3	120	83.16	1	2	3
4	0	22.80	2	1	3
4	15	23.50	2	1	3
4	30	23.80	2	1	3
4	45	47.00	2	1	3
4	60	84.92	2	1	3
4	75	87.24	2	1	3
4	90	80.30	2	1	3
4	105	80.16	2	1	3
4	120	75.39	2	1	3
5	0	28.80	2	2	3
5	15	28.60	2	2	3
5	30	29,00	2	2	3
5	45	50.06	2	2	3
5	60	74.42	2	2	3
5	75	81.16	2	2	3
5	90	82.69	2	2	3
5	105	57.59	2	2	3
5	120	62.87	2	2	3

AD - Anatomic Distribution
DT - Distribution over Time
SR - Speed and Rhythm
Source: Authors.

4. Conclusion

Dogs not supplemented with vitamin D do not have sufficient levels of this vitamin, being dependent on oral supplementation. This occurs due to the absence of 7–dihydrocholesterol in the vitamin D metabolization chain. The dose used in this study, of 1000IU/kg of vitamin D_3 (cholecalciferol) orally, had the potential to increase it to levels considered sufficient for dogs, changing, as expected, the serum levels of PTH, phosphorus and calcium, not changing red blood cells, ALP, hemoglobin, AST, ALT, urea, creatinine and hematocrit. It can also be concluded that the dose used did not have a toxic effect for the dogs supplemented, being considered safe to be used in dogs. However, the dose and duration of the treatment used did not change the myoclonus derived from distemper in dogs.

It is suggested, for the future, to perform studies with longer treatment periods with vitamin D_3, using different doses, with neuronal myelination evaluation methods, such as magnetic resonance imaging and evaluation of vitamin D concentration levels in the diet.

References

Abrita, R. R. (2015). Prevalência das alterações do metabolismo mineral e ósseo em pacientes com doença renal crônica em terapia renal substitutiva – Um estudo em centros de nefrologia da AMICEN. 95F. Dissertação (Mestrado em Medicina) – Setor de Saúde Brasileira, Universidade Federal de Juiz de Fora.

Almeida, R., Gonçalves, M., Viana, J., & Beirão, J. (1995). The semiology and classification of myoclonias. *Acta Médica Portuguesa*, 8(9), 523-7. http://dx.doi.org/10.20344/amp.2732

Barral, D., Barros, A. C. & Correia, R. P. A. (2007). Vitamina D: uma abordagem molecular. Pesquisa Brasileira em Odontopediatria e Clínica Integrada, 7(3). http://dx.doi.org/10.4034/pboci.v7i3.181

Bischoff-Ferrari, H. A., Shao, A., Dawson-Hughes, B., Hathcock, J., Giovannucci, E., & Willett, W. C. (2010). Benefit-risk assessment of vitamin D supplementation. *Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*, *21*(7), 1121–1132. https://doi.org/10.1007/s00198-009-1119-3

Castro, Luiz Claudio Gonçalves de. (2011). O sistema endocrinológico vitamina D. *Arquivos Brasileiros de Endocrinologia & Metabologia*, *55*(8), 566-575. https://doi.org/10.1590/S0004-27302011000800010

Davies, J., Heeb, H., Garimella, R., Templeton, K., Pinson, D., & Tawfik, O. (2012). Vitamin d receptor, retinoid x receptor, ki-67, survivin, and ezrin expression in canine osteosarcoma. *Veterinary medicine international*, *2012*, 761034. https://doi.org/10.1155/2012/761034

Embry, A. F., Snowdon, L. R., & Vieth, R. (2000). Vitamin D and seasonal fluctuations of gadolinium-enhancing magnetic resonance imaging lesions in multiple sclerosis. *Annals of neurology*, *48*(2), 271–272.

Fonseca, F. M. (2017). Concentração sérica de 25-hidroxivitamina D em cães saudáveis e como fator preditivo e prognóstico em cadelas com neoplasia mamária. 89f. Dissertação (Mestrado em Ciências Veterinárias) – Setor de Ciências Agrárias, Universidade Federal do Paraná.

Gerber, B., Hässig, M., & Reusch, C. E. (2003). Serum concentrations of 1,25-dihydroxycholecalciferol and 25-hydroxycholecalciferol in clinically normal dogs and dogs with acute and chronic renal failure. *American journal of veterinary research*, *64*(9), 1161–1166. https://doi.org/10.2460/ajvr.2003.64.1161

Gow, A. G., Else, R., Evans, H., Berry, J. L., Herrtage, M. E., & Mellanby, R. J. (2011). Hypovitaminosis D in dogs with inflammatory bowel disease and hypoalbuminaemia. *The Journal of small animal practice*, *52*(8), 411–418. https://doi.org/10.1111/j.1748-5827.2011.01082.x

Srivastav, Ajai K., Tiwari, P.R., Srivastav, S.K., Sasayama, Y., & Suzuki, N. (1997). Vitamin D3-induced calcemic and phosphatemic responses in the freshwater mud eel Amphipnous cuchia maintained in different calcium environments. *Brazilian Journal of Medical and Biological Research*, *30*(11), 1343-1348. https://doi.org/10.1590/S0100-879X1997001100014

Holick, M. F. (2007). Vitamin D deficiency pandemic: The healthful benefits of the d-lightful vitamin D. In: *Calcified Tissue International*. USA: SPRINGER, 16.

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., Murad, M. H., Weaver, C. M., & Endocrine Society (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *The Journal of clinical endocrinology and metabolism*, *96*(7), 1911–1930. https://doi.org/10.1210/jc.2011-0385

Holick M. F. (2016). Biological Effects of Sunlight, Ultraviolet Radiation, Visible Light, Infrared Radiation and Vitamin D for Health. *Anticancer research*, *36*(3), 1345–1356.

Holick M. F. (2017). The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. *Reviews in endocrine & metabolic disorders*, *18*(2), 153–165. https://doi.org/10.1007/s11154-017-9424-1

How, K. L., Hazewinkel, H. A., & Mol, J. A. (1994). Dietary vitamin D dependence of cat and dog due to inadequate cutaneous synthesis of vitamin D. *General and comparative endocrinology*, *96*(1), 12–18. https://doi.org/10.1006/gcen.1994.1154

Kraus, M. S., Rassnick, K. M., Wakshlag, J. J., Gelzer, A. R., Waxman, A. S., Struble, A. M., & Refsal, K. (2014). Relation of vitamin D status to congestive heart failure and cardiovascular events in dogs. *Journal of veterinary internal medicine*, *28*(1), 109–115. https://doi.org/10.1111/jvim.12239

Laws, E. J., Kathrani, A., Harcourt-Brown, T. R., Granger, N., & Rose, J. H. (2018). 25-Hydroxy vitamin D3 serum concentration in dogs with acute polyradiculoneuritis compared to matched controls. *The Journal of small animal practice*, *59*(4), 222–227. https://doi.org/10.1111/jsap.12791

Lips, P. (2006). Vitamin D physiology. *Progress in Biophysics and Molecular Biology*. 92(1):4-8. DOI: 10.1016/j.pbiomolbio.2006.02.016.

Looker, A. C., Johnson, C. L., Lacher, D. A., Pfeiffer, C. M., Schleicher, R. L., & Sempos, C. T. (2011). Vitamin D status: United States, 2001-2006. *NCHS data brief*, (59), 1–8.

Marques, Cláudia Diniz Lopes, Dantas, Andréa Tavares, Fragoso, Thiago Sotero, & Duarte, Ângela Luzia Branco Pinto. (2010). A importância dos níveis de vitamina D nas doenças autoimunes. *Revista Brasileira de Reumatologia*, *50*(1), 67-80. https://dx.doi.org/10.1590/S0482-50042010000100007

Nachreiner, R. F., Refsal, K. R., Rick, M. *et al*. (2014).Endocrinology reference ranges. *Diagnostic Center for Population & Animal Health*, Michigan State University, Lansing, Michigan, United States of America.

Norman A. W. (2012). The history of the discovery of vitamin D and its daughter steroid hormone. *Annals of nutrition & metabolism*, *61*(3), 199–206. https://doi.org/10.1159/000343104

Nunes, E. A. (2016). Impacto do exercício físico na hiperalgesia induzida pela administração repetida de morfina em ratos neonatos. 67f. Dissertação (Mestrado) – Ciências Biológicas, Universidade Federal do Rio Grande do Sul – UFRGS.

Orsini, H. & Bondan, E. F. (2008). Participação astrocitária na desmielinização do sistema nervoso central (SNC) de cães com cinomose**.** *Revista do Instituto de Ciências e Saúde*, 26(4);438-42.

Santos, M. H., Cabral, L. A. R., Martins, P. L. & Costa, P. P. C. (2016). Óbito de cadela imunossuprimida por cinomose nervosa: Relato de caso. *Revista Brasileira de Higiene e Sanidade Animal*, 10(1):117–133,

Selting, K. A., Sharp, C. R., Ringold, R., Thamm, D. H. & Backus, R. (2016). Serum 25-hydroxyvitamin D concentrations in dogs – correlation with health and cancer risk. *Veterinary and comparative oncology*, 14(3):295-305. https://doi.org/10.1111/vco.12101

Schenck, P. A., & Chew, D. J. (2008). Hypercalcemia: a quick reference. *The Veterinary clinics of North America. Small animal practice*, *38*(3), 449–viii. https://doi.org/10.1016/j.cvsm.2008.01.020

Sharp, C. R., Selting, K. A., & Ringold, R. (2015). The effect of diet on serum 25-hydroxyvitamin D concentrations in dogs. *BMC research notes*, *8*, 442. https://doi.org/10.1186/s13104-015-1360-0

Young, L. R., & Backus, R. C. (2016). Oral vitamin D supplementation at five times the recommended allowance marginally affects serum 25-hydroxyvitamin D concentrations in dogs. *Journal of nutritional science*, *5*, e31. https://doi.org/10.1017/jns.2016.23

Young, L. R., & Backus, R. C. (2017). Serum 25-hydroxyvitamin D_3 and 24*R*,25-dihydroxyvitamin D_3 concentrations in adult dogs are more substantially increased by oral supplementation of 25-hydroxyvitamin D_3 than by vitamin D_3. *Journal of nutritional science*, *6*, e30. https://doi.org/10.1017/jns.2017.8

Wacker, M., & Holick, M. F. (2013). Sunlight and Vitamin D: A global perspective for health. *Dermato-endocrinology*, *5*(1), 51–108. https://doi.org/10.4161/derm.24494

Wakshlag, J. J., Rassnick, K. M., Malone, E. K., Struble, A. M., Vachhani, P., Trump, D. L., & Tian, L. (2011). Cross-sectional study to investigate the association between vitamin D status and cutaneous mast cell tumours in Labrador retrievers. *The British journal of nutrition*, *106(1)*, S60–S63. https://doi.org/10.1017/S000711451100211X

Weidner, N., & Verbrugghe, A. (2017). Current knowledge of vitamin D in dogs. *Critical reviews in food science and nutrition*, *57*(18), 3850–3859. https://doi.org/10.1080/10408398.2016.1171202

Wheatley, V. R., & Sher, D. W. (1961). Studies of the lipids of dog skin. I. The chemical composition of dog skin lipids. *The Journal of investigative dermatology*, *36*, 169–170. https://doi.org/10.1038/jid.1961.29

Zhang, H. N., & Ko, M. C. (2009). Seizure activity involved in the up-regulation of BDNF mRNA expression by activation of central mu opioid receptors. *Neuroscience*, *161*(1), 301–310. https://doi.org/10.1016/j.neuroscience.2009.03.020