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The effects of age, sex, breed, diet, reproductive status and housing condition on the amounts of 25(OH) vitamin D in the serum of healthy dogs: Reference values

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Abstract

Background: Optimal amount of vitamin D for the proper functioning of the immune system is different from the required vitamin D amount for bones to prevent rickets. However, reports on vitamin D reference values in dogs are minimal, and there is still not enough information regarding the relationship between vitamin D and various variables such as disease, age, breed, diet type, and so on, as well as its relationship with haematological and serum biochemical parameters.

Obgectives: The present study aimed to determine reference values of 25(OH) Vit D in dogs and its concentration in different groups, categorized based on age, sex, breed, housing conditions, and diet, as well as 25(OH) Vit D relationship with hematology and serum biochemistry parameters.

Methods: In this study, 90 healthy dogs were selected to determine the reference value of 25 (OH) Vit D of serum after evaluating of their haematological and biochemical parameters to assess their general health. Dogs were divided into different groups according to above-mentioned variables. Serum 25 (OH) Vit D was subsequently measured by the ELISA method.

Results: The median concentration of 25 (OH) Vit D was 52.50 ng/ml with minimum and maximum amounts of 14.00 and 155.57 ng/ml, respectively. No significant difference was observed between 25 (OH) Vit D levels in the studied dogs regarding their different age, sex, breed, diet, housing condition, and reproductive status. Serum 25 (OH) Vit D concentration is directly correlated with the number of band neutrophils ($p < 0.05$). We also witnessed indirect correlations between serum 25 (OH) Vit D levels and the number of blood eosinophils and serum glucose ($p < 0.05$).

Conclusion: In the present study age, sex, breed, housing condition and age had no significant effects on the amounts of 25(OH) vitamin D. According to correlations of vitamin D with MCH, band and eosinophil numbers and glucose, vitamin D may have a role in erythropoiesis and leukocytes response and also in energy metabolism in dog.

KEYWORDS
25(OH) Vit D, canine, diet, ELISA, reference value

1 | INTRODUCTION

Following the development of industrial life, the importance of vitamin D was first suggested by Sir Edward Mellanby in 1918. He performed a study on some dogs feeding only on oatmeal for some time. After a while, the dogs showed skeletal disorders similar to rickets in children. He was able to cure these dogs by cod liver oil supplementation. It was later discovered that anti-rickets agent in fish oil is vitamin D (Hazewinkel et al., 2002; How et al., 1994; Mellanby et al., 2005). Since then, the metabolic pathways and physiological importance of vitamin D have been established (Borges et al., 2011; Clarke et al., 2021).

Studies on carnivores demonstrated that dogs and cats need higher amounts of vitamin D compared to other mammals (How et al., 1994). Scientists have found high cholesterol amounts in lipids of dog's skin, but none or very low levels of the intermediate metabolites, including 7-DHC were present (How et al., 1994; Wheatley et al., 1961). Therefore, it has been hypothesised that dogs' skin is inefficient in making vitamin D from ultraviolet radiation. These animals consequently depend solely on a diet to get the vitamin D they need (Fonseca et al., 2020). The liver of prey is the main source of vitamin D for carnivores, and food can regularly meet the need for vitamin D in these animals (How et al., 1994; Zafalon et al., 2020).

Once absorbed by the body and, or product in the skin, vitamin D is either stored in the adipose tissue or transported to the liver and then kidney to produce 25(OH) Vit D or calcidiol and 1,25-(OH)2 vitamin D or calcitriol, respectively. 25(OH) Vit D has a half-life of 10 to 21 days; therefore it is used as an indicator to assess body vitamin D levels (Mellanby et al., 2005; Weidner et al., 2017). The critical role of vitamin D in maintaining skeletal health and preventing disease led the National Research Council (NRC) to release a guideline in 1974 regarding sufficient amounts of vitamin D to meet the needs of dogs. Commercially available foods are primarily balanced for vitamin D content according to NRC's recommendation. Since commercial and natural foods consumed by dogs meet their need for vitamin D, it seems vitamin D deficiency rarely occurs in dogs (Hazewinkel et al., 2002; Weidner et al., 2017).

Vitamin D deficiency associated with various diseases in dogs. In the wide range of diseases such as gastrointestinal tract diseases (Clarke et al., 2021; Gow et al., 2011), various infectious diseases (Clarke et al., 2021; Rodríguez-Cortes 2017; Rosa et al., 2013), immune-mediated disorders (Mick et al., 2019), cardiovascular diseases (Parker et al., 2017), different type of neoplasia (Clarke et al., 2021; Weinder et al., 2017), diseases of the urinary tract (Galler et al., 2012; Parker et al., 2017), and inflammation, the amount of vitamin D was lower in diseased than healthy dogs. Also, the survival rate in hospitalised dogs with normal vitamin D levels was significantly higher (Jaffey et al., 2018). In addition, the response to treatment of atopic dermatitis with prednisolone was higher in dogs with normal levels of vitamin D (Kovalic et al., 2012). 'Chicken-and-egg situation' applies to the above-mentioned diseases with vitamin D deficiency. It is not clear which of the two events should be considered the cause and which one is the effect (Parker et al., 2017).

In cats and humans, the relationship between vitamin D levels and the RBC parameters and indices has been studied even though, in cats, no specific reason has been given to explain the relationship between vitamin D and anaemia (Titmarsh et al., 2017) and in humans, various reasons have been described (Alon et al., 2002; Atkinson et al., 2014; Aucella et al., 2001; Baccheta et al., 2014; Eloranta et al., 2009; Smith et al., 2015). A few researchers have studied the relationship between serum vitamin D concentrations and leukocyte populations in veterinary patients, and some relations were reported. According to their reports, hospitalised cats with a neutrophil count above the reference interval had lower vitamin D status (Titmarsh et al., 2017). In dogs with a chronic enteropathy, those with low serum 25(OH) Vit D concentrations typically had an inflammatory signature characterised by high monocyte and neutrophil counts with low lymphocyte numbers (Titmarsh et al., 2015). There are no studies on the relationship of vitamin D with haematological and biochemical serum variables in healthy dogs.

The optimal amount of vitamin D for the proper functioning of the immune system is different from the required vitamin D amount for bones to prevent rickets (Selting et al., 2016). However, reports on vitamin D reference values in dogs are minimal, and there is still not enough information regarding the relationship between vitamin D and various variables such as disease, age, breed, diet type and so on. The present study aimed to determine reference values of 25(OH) Vit D in dogs and its concentration in different groups, categorised based on age, sex, breed, housing conditions and diet, as well as 25(OH) Vit D relationship with haematology and serum biochemistry parameters. To the best of our knowledge, a few studies have been conducted to comprehensively investigate the factors affecting vitamin D levels in dogs. Also, it is the first report concerning vitamin D reference value in dogs in Iran.

2 | MATERIALS AND METHODS

2.1 | Study population

This study was performed on 90 healthy dogs from the northeast region of Iran (36° 17′ 60.00″ N, 59° 35′ 59.99″ E). The inclusion criteria include the animal's general health, non-pregnancy and non-lactation. Health measures include the history of surgery during the last six months, history of medication used within one month, history of vaccination and anti-parasitic drugs. To assess the health status, in addition to the general examination of the animal, its haematological and biochemical parameters were also considered. It was not possible to collect urine samples from all animals. The consent form and questionnaire related to the animal condition were filled out by the owners. The questionnaires asked for animal's age, gender, reproduction status, breed, housing condition, diet and health status. Based on this, 90 dogs were grouped into different categories, which are presented in Table 1. Age and breed were categorised according to published articles (Harvey, 2021; Mila et al., 2015). Due to the variety of diets, it was not possible to determine the exact amount of vitamin D in the diet of each group.

TABLE 1 The concentrations of 25(OH) Vit D (ng/ml) in total samples and various subgroups (median and 2.5–97.5 percentiles)

Group	Subgroups	Number	25(OH) D	*p* Value
Age	<1 year	12	41.8 (26.1–86.8)	0.140
	1–5 years	56	47.1 (15.2–93.7)	
	>5 years	22	55.6 (35.6–143.7)	
Gender	Male	40	48.2 (22.9–154.2)	0.922
	Female	50	49.7 (14.8–90.6)	
Reproductive status	Intact	61	49.4 (18.2–121.5)	0.595
	Spayed	29	47.3 (14.1–75.3)	
Breed	Large	47	47.9 (17.3–98.0)	0.394
	Small	43	50.2 (15.4–149.4)	
Diet	Commercial	8	44.1 (22.6–64.2)	0.496
	Home made	50	46.8 (17.5–94.8)	
	Mix	32	53.8 (14.0–115.40	
Housing condition	Outdoors	47	47.3 (17.3–98.0)	0.436
	Indoors	43	52.5 (15.4–149.4)	
Total	-	90	52.5 (15.4–149.8)	-

Subsequently, 10 ml of blood was taken from the jugular or brachial vein, 2.5 ml of the collected blood was transferred into EDTA-containing tubes and 7.5 ml was transferred to a tube without anticoagulant, in order to separate the serum. The samples were kept refrigerated and transported to the laboratory under appropriate conditions. Haematological evaluations were performed within less than an hour following sample collection.

CBC was performed using a veterinary haematology cell counter (Nihon Kohden, Celltac α, MEK-6450K, Tokyo, Japan) to evaluate the haematological status of the dogs. To separate the serum, collected blood was centrifuged at 1800 *g* for 10 min. The sera were then stored in a freezer at −20°C until further analysis. Furthermore, to ensure the health of the studied animals, biochemical parameters were initially measured by utilising commercial kits (Pars Azmoon, Tehran, Iran) by an autoanalyser (Biotecnica, BT 1500, Rome, Italy). Measured biochemical factors include albumin (ALB), blood urea nitrogen (BUN), creatinine (CRE), cholesterol (CHOL), triglyceride (TG), glucose (GLC), total bilirubin (TBIL), fibrinogen (FG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total calcium (Ca) and inorganic phosphorus (iP). The control serum (Randox Laboratories Ltd., Ardmore, UK) was used to check measurement accuracy. Any haematological and also biochemical abnormalities, which indicated Ca metabolism or kidney disorders were excluded the sampled dog from the study.

2.2 | Measurement of 25(OH) Vit D

Serum 25(OH) Vit D was measured using ELISA through commercial vitamin D diagnostic kits (Bioactiva diagnostic, Hamburg, Germany), by an ELISA washer and reader (Bio Tek, ELx-50, Winooski, USA, Bio Tek, ELx-800, Winooski, USA). Inter-assay CV, intra-assay CV and sensitivity of the commercial kit used are 6.95 %, 6.36% and 0.67 ng/ml, respectively.

2.3 | Statistical analyses

Data were statistically analysed using SPSS software version 20. The statistical distribution of the study population was initially examined by Shapiro–Wilk method. Since the statistical distribution of the studied population was not normal, non-parametric methods were used to determine the reference values and to examine the measured variable differences among studied groups. Therefore, the Mann–Whitney method was used to compare serum 25(OH) Vit D concentrations when two groups were compared, while Kruskal–Wallis method was used to compare 25(OH) Vit D concentrations where more than two groups were put into comparison. The correlations between serum 25(OH) Vit D concentration and haematological and biochemical parameters were determined using the Spearman's method. $p < 0.05$ was considered as significant for all analyses.

3 | RESULTS

In the present study, serum concentration of 25(OH) Vit D in sampled dogs has a median of 52.50 ng/ml, with 2.5% and 97.5% percentiles of 15.36 and 149.38 ng/ml, respectively. Furthermore, the minimum and maximum amounts of 25(OH) Vit D in this population were 14.00 and 155.57 ng/ml, respectively (Table 1).

No relationship was observed between serum 25(OH) Vit D levels and PCV, HB, RBC, MCV and MCHC. Nevertheless, serum 25(OH) Vit D concentration is directly correlated to MCH value ($p = 0.027$, Table 4). The amounts of haematological and serum biochemical variables of sampled dogs were also presented in Tables 2 and 3.

Our data also showed no relationship between serum 25(OH) Vit D levels and total leukocytes, neutrophils, lymphocytes, monocytes, basophils and platelets levels. However, serum 25(OH) Vit D concentration is directly correlated with the number of band neutrophils ($p = 0.016$). We also witnessed an indirect correlation between serum

TABLE 2 Median and percentiles (25 and 75) of haematological variables in sampled dogs

	PCV (%)	HGB (g/dl)	RBC (10^{12}/L)	MCV (fl)	MCH (pg)	MCHC (%)	WBC (10^9/L)	Neut (10^9/L)	Band (10^9/L)	Eos (10^9/L)	Lymph (10^9/L)	Mono (10^9/L)	Plt (10^9/L)
Median	42.1	16.15	6.58	64.5	24.6	38.0	11.15	7.43	0.08	0.31	2.50	0.38	265
25	39.1	14.78	6.06	61.6	23.2	36.5	8.90	5.63	0.0	0.15	1.74	0.24	191
75	45.5	17.40	7.19	66.7	25.3	38.9	13.40	9.57	0.13	0.66	3.36	0.71	345
RI*	35-57	12-19	4.95–7.87	66–77	21–26	32–36	5.0–14.1	2.9–12	0.0–0.45	0.0–1.3	0.4–2.9	0.1–1.4	211–621

*Used in authors university teaching hospital.

TABLE 3 Median and percentiles (25 and 75) of biochemical variables in sampled dogs

	Total Protein (g/dl)	Alb (g/dl)	BUN (mg/dl)	Cre (mg/dl)	Glu (mg/dl)	Chol (mg/dl)	TG (mg/dl)	BT (mg/dl)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Ca (mg/dl)	P (mg/dl)
Median	6.1	3.6	15.94	1.20	101	243	58	0.29	27	33	118	12.22	4.2
25	5.63	3.3	12.50	1.06	88	168	39	0.27	20	26	73	11.20	3.7
75	7.10	3.9	24.56	1.57	119	261	73	0.32	35	38	184	13.20	4.9
RI*	5.4–7.5	2.3–3.1	8–28	0.5–1.7	76–119	135–278	40–169	0.0–0.5	10–109	10–64	1–114	9.1–11.7	2.9–5.3

*Used in authors university teaching hospital.

TABLE 4 Correlation coefficient and *p* values between 25(OH) Vit D and haematological parameters

		PCV	HB	RBC	MCV	MCH	MCHC	WBC	Neut	Band	Eos	Lymph	Mono	Baso	Plt
25(OH) Vit D	Correlation coefficient	0.175	−0.0115	0.148	−0.192	0.233	−0.114	0.024	−0.141	0.253	−0.232	0.083	−0.066	0.047	0.026
	p Value	0.098	0.280	0.163	0.069	0.027*	0.286	0.821	0.186	0.016*	0.028*	0.437	0.537	0.659	0.811

*Significant correlation ($p < 0.05$).

25(OH) Vit D levels and the number of blood eosinophils ($p = 0.028$). There were no relationship between serum 25(OH) Vit D levels and TP, ALB, BUN, CRE, CHOL, TRIG, TBIL, ALT, AST, ALP, Ca and iP ($p > 0.05$). In the present study, we found that the serum concentration of 25(OH) Vit D was negatively related to the amount of serum Glu ($p = 0.018$) (Tables 4 and 5).

Investigation of the relationship between serum 25(OH) Vit D concentrations and gender showed that serum 25(OH) Vit D levels in male and female dogs were not significantly different ($p = 0.922$, Table 1). Serum 25(OH) Vit D levels in intact and spayed/neutered dogs were not significantly different ($p = 0.595$, Table 1). Serum 25(OH) Vit D concentrations were not significantly different in small versus large breeds ($p = 0.436$, Table 1). Serum amounts of 25(OH) Vit D in dogs kept indoors and outdoors were not significantly different ($p = 0.436$, Table 1).

In this study, most of dogs were 1–5 years old. The highest concentration of 25(OH) Vit D was observed in dogs over 5 years old, while the lowest concentration was seen in dogs aging between 1 and 5 years. However, this difference was insignificant among the three studied age groups ($p = 0.140$, Table 1). There were no significant differences between age groups in multiple comparison tests between group <1 year with group 1–5 years ($p = 0.652$), group <1 year with group >5 years ($p = 0.163$) and group 1–5 years with group >5 years ($p = 0.108$). The highest serum 25(OH) Vit D concentrations were seen in the group feeding on both home-made and commercial food (mix). However, the differences we witnessed among the three dietary groups were not statistically significant ($p = 0.496$).

4 | DISCUSSION

The concentration of 25(OH)D in the present study was 52.50 ng/ml (median). Our result differed from the values reported in other studies. As an example, in a study by Fonseca et al. (2020), serum 25(OH) Vit D concentration in dogs was less than 100 ng/ml, and 25(OH) Vit D levels in most of the dogs ranged between 25 and 100 ng/ml. Other research reported normal levels of 25(OH) Vit D between 9.5 and 249 ng/ml (Selting et al., 2016). In another survey, it has suggested that 25(OH) Vit D levels below 20–25 ng/ml may lead to bone growth disorders (Hazenwinkel et al., 2002). These differences in the amounts of measured 25(OH) Vit D in various studies might be due to different assessment methods while measuring vitamin D, because previous studies and also the result of present study indicated that diet did not affect the amounts of serum 25(OH) D. We performed complete blood

TABLE 5 Correlation coefficients and *p* values between 25(OH) Vit D and biochemical indicators

		Alb	BUN	Cre	Glu	Chol	TG	BT	ALT	AST	ALP	Ca	Pho
25(OH) Vit D	Correlation coefficient	0.127	0.079	−0.003	−0.253	−0.153	0.001	0.169	0.052	0.168	0.040	−0.128	0.440
	p value	0.242	0.463	0.975	0.018*	0.575	0.998	0.421	0.624	0.583	0.804	0.231	0.682

*Significant correlation ($p < 0.05$).

count and serum biochemistry from each dog for health assessment especially renal function as an important factor in serum vitamin D concentration. One of the limitation of our study was lack of urine analysis for all dogs. Thus, we cannot ensure that all sampled dogs did not have renal disease in primarily phase.

In the present study, there were no significant differences in 25(OH) Vit D concentrations groups' categorised based on gender and reproductive status. Similarly, in a study performed by Fonseca et al. (2020) no significant difference was observed between vitamin D levels and sex. However, in a study performed by Sharp et al. (2015), 25(OH) Vit D levels were higher in intact males dogs than in intact female dogs, while no significant difference was noticed between neutered males and spayed females. Our study was consistent with the results of several human studies that have been observed in the relationship between 25(OH) Vit D levels and sex (Câmara et al., 2019; Hagenau et al., 2009).

In the present study, 25(OH) Vit D amounts were the highest in dogs older than 5 years and the lowest in puppies less than 1 year old, but no significant difference was found in 25(OH) Vit D concentrations among the three studied age groups. However, in the studies performed by Hazenwinkel et al. (2002) and Fonseca et al. (2020) on dogs, this relationship was significant. They assumed this difference originates from high levels of growth hormone (GH) in puppies as well as lack of development in their gastrointestinal tract. High levels of GH may increase hydroxylation of 25(OH) Vit D levels and reduce its serum concentration. Additionally, during a study on humans, it was shown that there is no significant relationship between age and serum 25(OH) Vit D concentrations, which is consistent with our findings (Câmara et al., 2019).

In the present study, although 25(OH) Vit D was higher in dogs consuming a combination of homemade and commercial food compared to other groups, no significant difference was observed in terms of serum 25(OH) Vit D concentrations between the three groups. These results are consistent with the results of a study by Fonseca et al., since commercial foods often have balanced amounts of vitamin D. In the present study, it was expected that serum concentrations of 25(OH) Vit D in the dogs that consumed only commercial food was higher than other dogs, but no significant difference was observed. Because in the present study, different brands of commercial food were consumed by the dogs, it was not possible to follow up accurately in terms of the vitamin D content of the food.

Even though in Iran, large breeds of dogs are usually kept outdoors for guarding or herding dogs, and small breed dogs are often kept indoors, there was no significant difference between 25(OH) Vit D levels in these two groups. It is in accordance with recent report that confirmed the seasonal stability of 25(OH) Vit D concentrations in dogs (Hurst et al., 2020). This finding is an excellent evidence to support the hypothesis that the production of vitamin D in dogs skin is negligible and has no relation to radiation.

In the present study, correlations were found between Vit D and MCH levels. We cannot find any published data concerning the effects of Vit D on erythropoiesis in the dogs. In the present study, although vitamin D was directly related to MCH levels, there was no relationship between serum levels of this vitamin and other indicators of red blood cells. Thus, the exact cause and clinical importance of this correlation with MCH were not clear. It seems that more research concerning the role of vitamin D on the erythropoiesis of the dog will be needed.

In our study, positive and negative correlations were detected between Vit D and band and eosinophil numbers, respectively. A few researches have studied the relationship between serum vitamin D concentrations and leukocyte populations in either human or veterinary patients. In a recent study in dogs with chronic enteropathy, dogs with low serum 25(OH)D concentrations typically had high monocyte and neutrophil numbers and low lymphocyte numbers (Titmarsh et al., 2015). Another study that observed hospitalised cats with neutrophil bands had lower 25(OH)D values compared to cats without bands neutrophil. This difference approached significance ($p = 0.06$). There were no significant difference in 25(OH)D concentrations in cats with eosinophil, lymphocyte and monocyte counts above the upper RI compared with cats with leukocyte counts below the upper end of the RI (Titmarsh et al., 2017). The differences between our results with previous reports may be related to the health condition of dogs and also different behaviour of vitamin D in health and disease conditions.

In the present study, we found that the serum concentration of 25(OH) Vit D was negatively related to the amount of serum glucose. Vitamin D may be affected the synthesis and secretion of insulin by pancreatic beta cells in different ways. A previous study has shown that vitamin D can increase insulin secretion by affecting intracellular Ca concentration in pancreatic β cells and increasing insulin secretion (Borges et al., 2011). This increase revealed to increased uptake of glucose by muscle and adipose tissues and lowering serum Glucose concentration.

Although it is assumed that the amount of serum 25(OH) Vit D in dogs depends on the diet, in the present study, the diet had no effect on the amount of 25(OH) Vit D in the blood serum. Therefore, other unknown factors also affect the amount of 25(OH) Vit D in the dog's blood serum. Even though in the present study age, sex, breed, housing condition and age had no significant effects on the amounts of 25(OH) Vit D. According to correlations of vitamin D with MCH, band and eosinophil numbers and glucose, vitamin D may have a role in erythropoiesis and leukocytes response and also in energy metabolism in dog. Therefore, in future studies, it is better to determine the reference values of vitamin D concerning other vitamin D functions.

AUTHOR CONTRIBUTIONS

Kimia Alizadeh: data curation; investigation; validation; writing – review & editing. Saba Ahmadi: data curation; validation; writing – original draft; writing – review & editing. Ali Asghar Sarchahi: methodology; supervision; validation. Mehrdad Mohri: conceptualisation; data curation; formal analysis; funding acquisition; methodology; project administration; resources; supervision; validation; writing – review & editing

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received (3/48891). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

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REFERENCES

Alon, D. B., Chaimovitz, C., Dvilansky, A., Lugassy, G., Douvdevani, A., Shany, S., & Nathan, I. (2002). Novel role of 1, 25 (OH) 2D3 in induction of erythroid progenitor cell proliferation. *Experimental Hematology*, *30*(5), 403–409.

Atkinson, M. A., Melamed, M. L., Kumar, J., Roy, C. N., Miller III, E. R., Furth, S. L., & Fadrowski, J. J. (2014). Vitamin D, race, and risk for anemia in children. *The Journal of Pediatrics*, *164*(1), 153–158.

Aucella, F., Gatta, G., Vigilante, M., Scalzulli, R. P., Mantuano, S., Carotenuto, M., & Stallone, C. (2001). Calcitriol increases burst forming unit-erythroid (BFU-E) in vitro proliferation in chronic uremia. Synergic effect with DNA recombinant erythropoietin (rHu-Epo). *The Italian Journal of Urology and Nephrology*, *53*(1), 1–5.

Bacchetta, J., Zaritsky, J. J., Sea, J. L., Chun, R. F., Lisse, T. S., Zavala, K., Nayak, A., Wesseling-Perry, K., Westerman, M., Hollis, B. W., & Salusky, I. B. (2014). Suppression of iron-regulatory hepcidin by vitamin D. *Journal of the American Society of Nephrology*, *25*(3), 564–572.

Borges, M. C., Martini, L. A., & Rogero, M. M. (2011). Current perspectives on vitamin D, immune system, and chronic diseases. *Nutrition*, *27*(4), 399–404. https://doi.org/10.1016/j.nut.2010.07.022

Câmara, A. B., & Brandão, I. A. (2019). The relationship between vitamin D deficiency and oxidative stress can be independent of age and gender. *International Journal for Vitamin and Nutrition Research*, *91*(1–2), 108–123.

Clarke, K. E., Hurst, E. A., & Mellanby, R. J. (2021). Vitamin D metabolism and disorders in dogs and cats. *Journal of Small Animal Practice*, *62*(11), 935–947.

da Fonseca, F. M., Beltrame, O. C., Seixas, S. V., Laskoski, L. M., Félix, A. P., & Locatelli-Dittrich, R. (2020). Serum concentration of 25 (OH) vitamin D in healthy dogs: Factors as age, sex, and diet. *Comparative Clinical Pathology*, *29*, 697–703.

Eloranta, J. J., Zaïr, Z. M., Hiller, C., Häusler, S., Stieger, B., & Kullak-Ublick, G. A. (2009). Vitamin D3 and its nuclear receptor increase the expression and activity of the human proton-coupled folate transporter. *Molecular Pharmacology*, *76*(5), 1062–1071.

Galler, A., Tran, J. L., Krammer-Lukas, S., Höller, U., Thalhammer, J. G., Zentek, J., & Willmann, M. (2012). Blood vitamin levels in dogs with chronic kidney disease. *The Veterinary Journal*, *192*(2), 226–231.

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. *Journal of Small Animal Practice*, *52*(8), 411–418.

Hagenau, T., Vest, R., Gissel, T. N., Poulsen, C. S., Erlandsen, M., Mosekilde, L., & Vestergaard, P. (2009). Global vitamin D levels in relation to age, gender, skin pigmentation and latitude: An ecologic meta-regression analysis. *Osteoporosis International*, *20*(1), 133–40.

Harvey, N. D. (2021). How old is my dog? Identification of rational age groupings in pet dogs based upon normative age-linked processes. *Frontiers in Veterinary Science*, 321.

Hazewinkel, H. A. W., & Tryfonidou, M. A. (2002). Vitamin D3 metabolism in dogs. *Molecular and Cellular Endocrinology*, *197*(1-2), 23–33.

How, K. L., Hazewinkel, H. A. W., & 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.

Hurst, E. A., Homer, N. Z., Gow, A. G., Clements, D. N., Evans, H., Gaylor, D., Campbell, S., Handel, I., & Mellanby, R. J. (2020). Vitamin D status is seasonally stable in northern European dogs. *Veterinary Clinical Pathology*, *49*(2), 279–291.

Jaffey, J. A., Backus, R. C., McDaniel, K. M., & DeClue, A. E. (2018). Serum vitamin D concentrations in hospitalized critically ill dogs. *PLoS One*, *13*(3), e0194062.

Kovalik, M., Thoday, K. L., Berry, J., van den Broek, A. H., & Mellanby, R. J. (2012). Prednisolone therapy for atopic dermatitis is less effective in dogs with lower pretreatment serum 25-hydroxyvitamin D concentrations. *Veterinary Dermatology*, *23*(2), 125–e28.

Mellanby, R. J., Mee, A. P., Berry, J. L., & Herrtage, M. E. (2005). Hypercalcaemia in two dogs caused by excessive dietary supplementation of vitamin D. *Journal of Small Animal Practice*, *46*(7), 334–338.

Mick, P. J., Peng, S. A., & Loftus, J. P. (2019). Serum vitamin D metabolites and CXCL10 concentrations associate with survival in dogs with immune mediated disease. *Frontiers in Veterinary Science*, *6*, 247.

Mila, H., Grellet, A., Feugier, A., & Chastant-Maillard, S. (2015). Differential impact of birth weight and early growth on neonatal mortality in puppies. *Journal of Animal Science*, *93*(9), 4436–4442.

Parker, V. J., Rudinsky, A. J., & Chew, D. J. (2017). Vitamin D metabolism in canine and feline medicine. *Journal of the American Veterinary Medical Association*, *250*(11), 1259–1269.

Rodríguez-Cortes, A., Martori, C., Martinez-Florez, A., Clop, A., Amills, M., Kubejko, J., Nadal, J. M., & Alberola, J. (2017). Canine leishmaniasis progression is associated with vitamin D deficiency. *Scientific Reports*, *7*(1), 1–10.

Rosa, C. T., Schoeman, J. P., Berry, J. L., Mellanby, R. J., & Dvir, E. (2013). Hypovitaminosis D in dogs with spirocercosis. *Journal of Veterinary Internal Medicine*, *27*(5), 1159–1164.

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.

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*(1), 1–6.

Smith, E. M., & Tangpricha, V. (2015). Vitamin D and anemia: Insights into an emerging association. *Current Opinion in Endocrinology, Diabetes, and Obesity*, 22(6), 432.

Titmarsh, H. F., Gow, A. G., Kilpatrick, S., Cartwright, J. A., Milne, E. M., Philbey, A. W., Berry, J., Handel, I., & Mellanby, R. J. (2015). Low vitamin D status is associated with systemic and gastrointestinal inflammation in dogs with a chronic enteropathy. *PLoS One*, *10*(9), e0137377.

Titmarsh, H. F., Cartwright, J. A., Kilpatrick, S., Gaylor, D., Milne, E. M., Berry, J. L., Bommer, N. X., Gunn-Moore, D., Reed, N., Handel, I., & Mellanby, R. J. (2017). Relationship between vitamin D status and leukocytes in hospitalised cats. *Journal of Feline Medicine and Surgery*, *19*(4), 364–369.

Weidner, N., & Verbrugghe, A. (2017). Current knowledge of vitamin D in dogs. *Critical Reviews in Food Science and Nutrition*, *57*(18), 3850–3859.

Wheatley, V. R. (1961). Studies of the lipids of dog skin. I. The chemical composition of dog skin lipids. *Journal of Investigative Dermatology*, *36*, 169–70.

Zafalon, R. V., Risolia, L. W., Pedrinelli, V., Vendramini, T. H., Rodrigues, R. B., Amaral, A. R., Kogika, M. M., & Brunetto, M. A. (2020). Vitamin D metabolism in dogs and cats and its relation to diseases not associated with bone metabolism. *Journal of Animal Physiology and Animal Nutrition*, *104*(1), 322–342.

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