Short Paper

Serum concentration of 25-hydroxyvitamin D in apparently healthy cats regarding age, gender, breed, diet type, reproductive status, and housing condition

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Abstract

Background: Optimal vitamin D levels for an effective role in immune function and rickets prevention are thought to vary, but have not yet been definitively determined. Reports on reference values of 25-hydroxyvitamin D (25(OH)D) in cats are limited. **Aims:** The study provides information about serum 25(OH)D values in cats with different age, gender, breed, diet type, reproductive status, housing condition, and also the relationship between these levels and various hematological and biochemical parameters. **Methods:** Clinically healthy cats (88) were included in the study. Physical examination and assessment of hematological and biochemical parameters were performed on cats in order to confirm their health status. Reference value of serum 25(OH)D was assayed by ELISA method and the effects of age (under six months and above six months), gender, breed, diet (only commercial diet, only homemade food, mixture of commercial and homemade food), reproduction status, and housing conditions on serum 25(OH)D was determined. **Results:** The median, 2.5% and 97.5% of 25(OH)D in sampled cats were 19.74 ng/ml, 3.12 ng/ml, and 92.1 ng/ml, respectively. Serum 25(OH)D concentration was lower when homemade diet was used compared to commercial and mixed diets as well as in cats under six months of age compared to older cats. **Conclusion:** Diet type and age can affect serum 25(OH)D levels in healthy cats while other parameters had no significant effects.

Key words: 25(OH)D, Feline, Diet, ELISA, Reference value

Introduction

Vitamin D is a conditional nutrient in many species that is synthesized if the skin's 7-dihydro cholesterol (7-DHC) is exposed to sunlight (Morris, 1999). The cat has numerous nutritional peculiarities, including inefficiency in synthesizing vitamin D when exposed to sunlight (Morris, 2002a). Studies have shown that even though cats possess adequate amounts of 7-DHC in their skin, it is reduced by the 7-DHC delta reductase enzyme before being used as a precursor for vitamin D synthesis (Cline, 2012). Therefore, cats exclusively rely on dietary vitamin D intake. During their adaptive evolution, these animals have become obligatory carnivores to feed on prey animals (Morris, 2002b). Since the main storage organ for vitamin D is the liver, thus the continuous intake of prey liver prevents vitamin D deficiency in cats (Morris, 2002b; Parker *et al*., 2017; Zafalon *et al*., 2020). However, homemade meals not formulated by the veterinarian can cause deficiency because the amount of vitamin D in homemade meals is not regulated based on the daily requirements of a cat (Hurst *et al*., 2020). Another difference in cats concerning to vitamin D metabolism is the higher serum vitamin D3 epimers C3 than other species. C3 epimerization is a protective mechanism for cats that make them highly resistant to vitamin D toxicity (Hurst *et al*., 2020).

Cats generally receive vitamin D from two different sources: cholecalciferol, which is primarily obtained from animal food sources, and ergocalciferol from a plant-based diet (Parker *et al*., 2017; Sprinkle *et al*., 2018). In general, the bioavailability of vitamin D2 in cats is lower than other species. In diets with D2 and D3 content, cats consume vitamin D3 to produce 25(OH)D

metabolites approximately 31% more efficiently than vitamin D2 (Sprinkle *et al*., 2018). This may be due to the lower affinity of vitamin D binding protein for vitamin D2 in cat (Hurst *et al*., 2020). Thus in cat nutrition, commercial diets are generally supplemented with vitamin D3.

The amount of vitamin D requirement depends on the amount of calcium (Ca), phosphorus (P), and Ca/P ratio in the diet, as well as the physiological condition of the cat (Cline, 2012). Commercial cat foods are fortified with various ingredients, including organ meats, oily fish, and vitamin D3 supplements (Parker *et al*., 2017). According to the American Association of Food Control Officials, the vitamin D, Ca, and P requirements for cats are as follows: 1) growth: vitamin D 750 unit/dry matter (IU/DM), calcium 1% DM, phosphorus 0.8% DM, and Ca: P ratio 1.2:1, 2) Maintenance: vitamin D 500 IU/DM, calcium 0.6% DM, phosphorus 0.5% DM, and Ca: P ratio 1.2:1 (Cline, 2012).

Vitamin D and 25(OH)D have widespread roles in the body (as a vitamin and also as a hormone), including Ca and P homeostasis, cell proliferation and differentiation, hormone secretion, and regulation of immune-system (Ahmadi and Mohri, 2021). 25(OH)D has a half-life of 10 to 21 days; therefore, it is used as an indicator to assess body vitamin D content (Titmarsh *et al*., 2015).

In general, little is known about the 25(OH)D reference values in cats. In addition, limited research is being conducted to investigate the relationship between 25(OH)D level and biochemical and hematological parameters, as well as age, sex, breed, diet, and housing conditions. According to the author's knowledge, future studies could be performed to comprehensively investigate the factors affecting the serum's 25(OH)D amount. This is the first study conducted in Iran to establish a reference value for 25(OH)D in cats and its relations with other dichomatous, hematological, and serum biochemical variables.

Materials and Methods

Ethics 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 (3/53160). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

Study groups

The methods of study were approved by our institutional ethical committee (3/53160). At the first step, 90 cats from different breeds which were admitted to various veterinary clinics in the city, for checking or castration were selected. The inclusion criteria for this study were general health, non-pregnancy, and no breastfeeding. Healthy cats were included in the research and subsequently underwent additional hematological and serum biochemical tests. The owner of the animal completed an informed consent form and a questionnaire regarding the animal's condition. The questionnaire included age, sex, breed, housing conditions, diet, and health measures. Then, 88 selected cats were allocated into different groups as follows and two cats were ruled out from the study due to the lack of inclusion criteria:

1. Age: Most cats which were assigned to this study aged between six months and two years old. Cats over the seven years old were not found. Cats were allocated into two age groups: under six months (n=21) and above six months (n=76).
2. Sex: A total of 21 male cats and 67 female cats were included in this study.
3. Reproductive status: There were 25 neutered or spayed cats and 63 intact cats.
4. Breed: 76 domestic short-haired and 12 domestic long-haired cats.
5. Diet: Cats were divided into three diet groups: (A) cats that exclusively were fed by commercial diet (n=37), (B) Cats that exclusively were fed by homemade food (n=30), and (c) Cats that were fed with commercial and homemade food (n=21).
6. Housing conditions: Of all the cats in the study, 61 cats were kept indoors, and 27 cats were kept outdoors.

Health measures included negative surgery history during the last six months, negative medication history, vaccination, and anthelmintic drug use within the last month.

Sample collection

Five to 10 ml of blood was collected from the jugular or brachial vein of dogs, depending on the body condition score. A portion of the collected blood (2.5 ml) was transferred to the EDTA-containing tubes. The remaining was transferred to a plain tube without anticoagulant to separate the serum and kept at the optimum condition for further analysis (on ice and at 4°C). Complete blood cells count (CBC) was performed by Nihon Kohden Veterinary Cell Counter (Celltac α, MEK-6450K, Japan). The collected blood was centrifuged at 1800 g for 10 min to separate the serum. Sera were stored in a freezer at -20°C until further analysis. Biochemical assays were performed using commercially available kits (Pars Azmoun, Tehran, Iran) and an automated biochemical analyzer (Biotechnica, BT 1500, Rome, Italy) and included determination of total calcium, phosphorus, magnesium, urea, and creatinine concentrations. In addition, a urinalysis was performed on void urine sample from all sampled cats.

Measurement of 25(OH)D

A commercially available 25(OH)D competitive-saturated ELISA kit which was previously validated for measuring 25(OH)D in cats in our laboratory (Pishgaman Sanjesh Isatis, Tehran, Iran) was used for measuring 25(OH)D in cat serum. The inter-assay CV and intra-assay CV of these kits using a fully nested ANOVA method are 9.53% and 14.78%, respectively. The lower and higher limit of detection and sensitivity are 4, 120,

and 1.8 ng/ml, respectively. Specificity of kit for measuring 25(OH)D3, 25(OH)D2, vitamin D3, and vitamin D2 are 100, 83, <0.1, and <0.1%, respectively.

ELISA washer and reader (Bio Tek, ELx 50 and ELx 800, Winooski, USA) were used to wash the plate and read the absorbance of the wells.

Statistical analysis

After examining the statistical distribution of 25(OH)D in the sampled population by SPSS software version 20 and the Shapiro-Wilk method, it was found that the distribution of 25(OH)D is not normal. Thus, a non-parametric method was used to determine the reference values. In order to determine the amount of 25(OH)D in the entire population and different groups, the median was used as the central index and the percentiles of 2.5% and 97.5% as the lower and upper limits. The Mann-Whitney method was used to compare the amount of 25(OH)D between two groups based on age, sex, reproductive status, breed, and housing condition. The Kruscal-Wallis one way analysis of variance was used to compare 25(OH)D levels among more than two groups (e.g. diet groups). Finally, the correlation was determined between the amounts of 25(OH)D and hematological and serum biochemical indices by the Spearman's correlation method. In all comparisons, $P<0.05$ was considered as the level of significance.

Results

The reference values of serum 25(OH)D

The median and 2.5%-97.5% percentile of 25(OH)D in 88 sampled cats were 19.74 ng/ml and 3.12 ng/ml - 92.1 ng/ml, respectively. The minimum and maximum levels of 25(OH)D in this population were 2.86 ng/ml and 102.58 ng/ml, respectively. All results are presented in Table 1.

Serum 25(OH)D concentration was significantly lower when cats were fed with homemade compared to commercial or mixed diets and in cats under six months in age compared to older cats. However, no significant difference was observed in 25(OH)D between groups with other grouping factors (Table 1).

Correlation between serum 25(OH)D and hematological and some biochemical parameters

There were no statistically significant relations between 25(OH)D concentrations and WBC, PLT, PCV, HB, RBC, MCH, MCHC, RDW, TP, and MCV amounts and Ca, P, Mg, urea, and creatinine concentrations.

Discussion

In the present study, the median serum 25(OH)D concentration was 19.74 ng/ml. This value differed from reported results in some previous studies; for instance, in the study by Lalor *et al*. (2012) the 25(OH)D concentration in 36 healthy cats was determined to be 49 ng/ml. In another research conducted by Titmarsh *et al*. (2015) in 20 healthy cats, the mean value of 25(OH)D was 44.7 ng/ml. In the study by Sprinkle *et al*. (2018), the mean value of 25(OH)D in cats supplemented with vitamin D was reported to be 45.6 ± 10.3 ng/ml. We believe there are various reasons for these controversies in the 25(OH)D levels. First, the method of determining vitamin D levels was different for each study. Second, pet diets and commercial food brands may also be influential. In addition, some cats took vitamin D supplements, which increased the average amount of vitamin D. Third, the number of cats used in various studies was different and caused the 25(OH)D concentration difference between reports.

In the present study, the concentration of 25(OH)D was lower in the cats under six months than older group. These results are consistent with the findings of Pineda *et al*. (2013), who showed that calcidiol concentration increases as the kittens grow. In another study, the concentrations of calcidiol were measured in kittens from 7 to 22 weeks of age in six groups with different amounts

Table 1: The concentrations of 25(OH)D (ng/ml) in various subgroups [median (2.5-97.5 percentiles)] of cats

Group	Subgroups	Number	25(OH)D	P-value
Age	<6 months	12	10.31 (2.86-20.48)[a]	0.010
	>6 months	76	21.05 (5.29-93.35)[b]	
Gender	Male	21	19.57 (2.91-95.59)[a]	0.721
	Female	67	19.9 (3.8-29.24)[a]	
Reproductive status	Intact	63	17.6 (2.9-96.59)[a]	0.070
	Spayed	25	25.40 (7.16-38.53)[a]	
Breed	Long hair	12	27.26 (5.41-40.37)[a]	0.588
	Short hair	76	18.90 (2.92-93.35)[a]	
Diet	Commercial	21	27.92 (10.04-44.02)[a]	0.006
	Homemade	30	10.68 (2.86-26.50)[b]	
	Mix	37	20.87 (3.8-33.75)[a]	
Housing condition	Outdoors	27	24.57 (5.46-36.95)[a]	0.196
	Indoors	61	18.48 (2.90-97.09)[a]	

The subgroups with different superscripts were significantly different ($P<0.05$)

of cholecalciferol in their diet. The amounts of calcidiol significantly increased with age in groups that received a higher amount of cholecalciferol in their diet (Morris *et al*., 1999). Conversely, according to the study conducted by Ware *et al*. (2020), there is a negative relationship between age and 25(OH)D3 concentration in normal cats. It should be mentioned that the range of age of normal cats in their study was 1 to 23 years (Ware *et al*., 2020). Several reasons may result in an increase in the amount of 25(OH)D with age, including: milk is a poor source of vitamin D, and the newborn intestinal system is not well developed to absorb vitamin D (Fahey *et al*., 2008). Notably, there were newly weaned kittens in the group under six months old in our study who showed relatively low vitamin D levels. Growth hormone levels are very high at young ages. High growth hormone levels can increase the hydroxylation of 25(OH)D and decrease its serum concentration (Hazewinkel and Tryfonidou, 2002; Tryfonidou *et al*., 2003). It seems that the immune system is more active at young ages and consumes more vitamin D that consequently reduces 25(OH)D levels.

In the present study, 25(OH)D concentrations were not significantly different between male and female cats. Girard *et al*. (2010), also reported no significant difference in vitamin D amounts between male and female groups (Girard *et al*., 2010). These findings were also consistent with the results of another previous report (Ware *et al*., 2020). Therefore, sex hormones do not seem to influence the levels of 25(OH)D in cats. Accordingly, we found no significant difference between the amounts of 25(OH)D in the spayed/neutered and intact cats.

In our study, we did not observe significant difference in 25(OH)D levels between DSH and DLH breeds of cats. It seems that fur thickness does not affect 25(OH)D levels, especially by considering the fact vitamin D is not produced in cat skin (Cline, 2012). In agreement with the present study, Ware *et al*. (2020) suggested no significant difference between different breeds of cats concerning vitamin D concentrations. In contrast, Girard *et al*. (2010) suggested a significant difference in 25(OH)D levels between different breeds of cats.

There was no significant difference in 25(OH)D levels between cats kept outdoors and indoors in the present study. Our study's results are in agreement with a previous study on kittens (Morris, 1991). This result confirms that cats' skins are inefficient in vitamin D synthesis.

As expected, group that consumed only homemade food showed significantly lower 25(OH)D levels than those who consumed commercial or a combination of commercial and homemade diets, while cats in the latter groups showed statistically the same levels of serum 25 (OH)D. As far as the authors know, similar studies have yet to be performed on cats. Our results are partly in agreement with those of da Fonseca *et al*. (2020) in dogs who also reported no difference between two types of diet: commercial food (40.6 ng/ml) and the combination of commercial and homemade food (36.0 ng/ml). It should be noted that homemade foods are generally not balanced, and this is the cause of the low amount of vitamin D in the group fed only with homemade diet. Since commercial foods are balanced in terms of micronutrients, as expected, the highest amount of vitamin D was in the group that consumed only commercial foods. In this study, we could not evaluate the amount of vitamin D in foods of different diet groups because different brands of commercial foods or different homemade foods were used.

No correlations between serum 25(OH)D concentration and hematological and biochemical parameters were observed in this study. It has been reported that in hospitalized cats, those with neutrophilia show lower serum 25(OH)D concentrations than cats with neutrophil concentrations below the upper limit of the reference interval (RI). However, there were no significant differences 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). A significant difference was reported between cats with anemia and healthy ones. Red blood cell count and mean corpuscular volume were negatively correlated with serum 25(OH)D concentrations (Titmarsh *et al*., 2020). In a recent study in dogs, serum 25(OH)D concentration was reported to be directly correlated with the amounts of band neutrophils and also indirect correlations between serum 25(OH)D levels and the number of blood eosinophils and serum glucose was found (Alizadeh *et al*., 2022). The differences between our results with previous reports may be related to the health condition and the different behavior of vitamin D in health and disease conditions in different species.

The prominent role of the active form of vitamin D is to increase serum calcium concentrations by increasing intestinal absorption and renal reabsorption of calcium (Titmarsh *et al*., 2017). Although in the present study, no correlation was found between 25(OH)D and calcium concentrations, a previous study revealed that 25(OH)D level decreases with higher dietary Ca levels (Paßlack *et al*., 2016).

In conclusion, we found that serum 25(OH)D concentration was lower when homemade compared to commercial diets were consumed and in cats under six months compared to older cats. However, no significant difference was observed between 25(OH)D for other grouping factors. Therefore, age-specific reference value must be considered for any interpretation of vitamin D amounts in the cat.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

Ahmadi, S and Mohri, M (2021). New outlook to vitamin D functions in dairy cows: non-classical roles. Iran. J. Vet. Sci. Techno., 13: 1-11.

Alizadeh, K; Ahmadi, S; Sarchahi, AA and Mohri, M (2022). 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. Vet. Med. Sci., 8: 2360-2366.

Cline, J (2012). Calcium and vitamin D metabolism, deficiency, and excess. Top. Comp. Anim. Med., 27: 159-164.

da Fonseca, FM; Beltrame, OC; Seixas, SV; Laskoski, LM; Félix, AP and Locatelli-Dittrich, R (2020). Serum concentration of 25 (OH) vitamin D in healthy dogs: factors as age, sex, and diet. Comp. Clin. Pathol., 29: 697-703.

Fahey, JrGC; Barry, KA and Swanson, KS (2008). Age-related changes in nutrient utilization by companion animals. Annu. Rev. Nutr., 28: 425-445.

Girard, N; Servet, E; Hennet, P and Biourge, V (2010). Tooth resorption and vitamin D3 status in cats fed premium dry diets. J. Vet. Dent., 27: 142-147.

Hazewinkel, HA and Tryfonidou, MA (2002). Vitamin D3 metabolism in dogs. Mol. Cell. Endocrinol., 197: 23-33.

Hurst, EA; Homer, NZ and Mellanby, RJ (2020). Vitamin D metabolism and profiling in veterinary species. Metabolites. 10: 371.

Lalor, SM; Mellanby, RJ; Friend, EJ; Bowlt, KL; Berry, J and Gunn-Moore, D (2012). Domesticated cats with active mycobacteria infections have low serum vitamin D (25 (OH) D) concentrations. Trans. Emerg. Dis., 59: 279-281.

Morris, JG (1999). Ineffective vitamin D synthesis in cats is reversed by an inhibitor of 7-dehydrocholestrol-Δ7-reductase. J. Nutr., 129: 903-908.

Morris, JG (2002a). Cats discriminate between cholecalciferol and ergocalciferol. J. Anim. Physiol. Anim. Nutr., 86: 229-238.

Morris, JG (2002b). Idiosyncratic nutrient requirements of cats appear to be diet-induced evolutionary adaptations. Nutr. Res. Rev., 15: 153-168.

Morris, JG; Earle, KE and Anderson, PA (1999). Plasma 25-hydroxyvitamin D in growing kittens is related to dietary intake of cholecalciferol. J. Nutr., 129: 909-912.

Paßlack, N; Schmiedchen, B; Raila, J; Schweigert, FJ; Stumpff, F; Kohn, B; Neumann, K and Zentek, J (2016). Impact of increasing dietary calcium levels on calcium excretion and vitamin D metabolites in the blood of healthy adult cats. PLoS One. 11: e0149190.

Parker, VJ; Rudinsky, AJ and Chew, DJ (2017). Vitamin D metabolism in canine and feline medicine. JAVMA, 250: 1259-1269.

Pineda, C; Aguilera-Tejero, E; Guerrero, F; Raya, AI; Rodriguez, M and Lopez, I (2013). Mineral metabolism in growing cats: changes in the values of blood parameters with age. J. Fel. Med. Surg., 15: 866-871.

Sprinkle, MC; Hooper, SE and Backus, RC (2018). Previously undescribed vitamin D C-3 epimer occurs in substantial amounts in the blood of cats. J. Fel. Med. Surg., 20: 83-90.

Titmarsh, HF; Cartwright, JA; Kilpatrick, S; Gaylor, D; Milne, EM; Berry, JL; Bommer, NX; Gunn-Moore, D; Reed, N; Handel, I and Mellanby, RJ (2017). Relationship between vitamin D status and leukocytes in hospitalised cats. J. Fel. Med. Surg., 19: 364-369.

Titmarsh, HF; Lalor, SM; Tasker, S; Barker, EN; Berry, J; Gunn-More, D and Mellanby, RJ (2015). Vitamin D status in cats with feline immunodeficiency virus. Vet. Med. Sci., 1: 72-78.

Titmarsh, HF; Woods, GA; Cartwright, JA; Kilpatrick, S; Gaylor, D; Berry, J; Gow, A; Bommer, NX; Gunn-Moore, D; Handel, I and Mellanby, RJ (2020). Low vitamin D status is associated with anaemia in hospitalised cats. Vet. Rec., 187: e6.

Tryfonidou, MA; Holl, MS; Stevenhagen, JJ; Buurman, CJ; Deluca, HF; Oosterlaken-Dijksterhuis, MA; Van Den Brom, WE; Van Leeuwen, JP and Hazewinkel, HA (2003). Dietary 135-fold cholecalciferol supplementation severely disturbs the endochondral ossification in growing dogs. Domes. Anim. Endocrinol., 24: 265-285.

Ware, WA; Freeman, LM; Rush, JE; Ward, JL; Makowski, AJ and Zhang, M (2020). Vitamin D status in cats with cardiomyopathy. J. Vet. Inter. Med., 34: 1389-1398.

Zafalon, RV; Risolia, LW; Pedrinelli, V; Vendramini, TH; Rodrigues, RB; Amaral, AR; Kogika, MM and Brunetto, MA (2020). Vitamin D metabolism in dogs and cats and its relation to diseases not associated with bone metabolism. J. Anim. Physiol. Anim. Nutr., 104: 322-342.