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Dysregulated serum concentrations of fat-soluble vitamins in dogs with chronic enteropathy

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Abstract

Background: In inflammatory bowel disease (IBD) of humans, nutrient malabsorption can result in fat-soluble vitamin deficiency, especially of vitamin D. In veterinary species, decreased concentrations of vitamin D are relatively common in dogs with chronic enteropathy (CE), but data on the status of other fat-soluble vitamins (FSVs) is lacking.

Objectives: Determine the serum concentrations of retinol, vitamin D, and α-tocopherol in dogs with CE compared with healthy dogs and compare clinical, clinicopathologic variables between CE and healthy dogs to detect associations with decreased FSVs concentrations.

Animals: Eighteen client-owned dogs with CE and 33 healthy dogs.

Methods: Serum 25-hydroxyvitamin D (25[OH]D), serum retinol and α-tocopherol concentrations were compared between groups. Correlations and multiple regression modeling were used to examine the relationship between serum 25(OH)D, retinol, and α-tocopherol concentrations and clinical and clinicopathological variables.

Results: Dogs with low serum albumin concentrations were more likely to have lower 25(OH)D concentrations than dogs with normal serum albumin concentration. Dogs with CE had higher serum concentrations of retinol, and variable α-tocopherol concentrations. The cause of these dysregulated vitamin concentrations is unclear and requires further study.

Conclusion and Clinical Importance: Dogs with severe forms of CE should be monitored for decreased concentrations of 25(OH)D. Additional studies are needed to evaluate the clinical relevance and the possible benefit of vitamin D supplementation in these patients.

KEYWORDS
inflammatory bowel disease, retinol, vitamins D, α-tocopherol

1 | INTRODUCTION

Vitamins A, D, and E are fat-soluble vitamins (FSVs), essential nutrients that ensure optimal growth, reproduction and sustain many other

Abbreviations: 25(OH)D, serum 25 hydroxyvitamin D; BCS, body condition score; CCECAI, canine chronic enteropathy activity index; CE, chronic enteropathy; CIBDAI, canine inflammatory bowel disease activity index; cPLI, canine pancreatic lipase immunoreactivity; cTLI, canine trypsin-like immunoreactivity; FSVs, fat-soluble vitamins; IBD, inflammatory bowel disease.

life functions. The FSVs have important effects in regulation of immune responses.[1] They cannot be synthesized by dogs in sufficient amounts, and thus need to be provided in the diet. Fat soluble vitamins have a competitive interaction, and a common absorption pathway has been suggested.[2] Diseases that affect dietary fat absorption, such as intestinal lymphangiectasia or long-standing inflammatory bowel disease (IBD) and chronic enteropathy (CE) could result in FSV deficiency.[3-5]

In humans, vitamin D plays a direct role in immune tolerance and in the maintenance of the intestinal barrier. Its concentrations are inversely related to the severity of intestinal inflammation.[6] Furthermore, vitamin D deficiency has been associated with increased risk of hospitalization, surgical intervention, and mortality in people with IBD. Vitamin A and D concentrations are integrally related. Interestingly, the interaction of circulating vitamin A with the vitamin D nuclear receptor could decrease the risk of death in patients with vitamin D deficiency.[7] Additionally, vitamin A plays a role in the immune response, controlling immunoglobulin A response and intervening in the regulation of phagocytic activity. Vitamin A deficiency has been associated with an increase of inflammatory cytokines and severe inflammation.[8,9] As an antioxidant, vitamin E improves immune function by acting as a scavenger of free radicals and suppressing the expression of proinflammatory cytokines.[10] A recent study in humans determined that the administration of exogenous α-tocopherol led to remission of clinical signs in 64% of patients with ulcerative colitis.[4] Therefore, decreased serum concentrations of these FSVs theoretically could contribute to the dysregulation of immune responses and worsening of intestinal inflammation.[4]

Moreover, a growing body of evidence suggests that serum 25(OH)D concentrations are decreased in dogs[11-16] and cats[17] with CE. Studies have shown that vitamin D status in dogs with CE might be associated with systemic and local inflammation.[13,16] On the other hand, vitamins A and E have received far less attention than vitamin D in veterinary literature. Despite the lack of evidence for FSV deficiencies, supplementation of FSVs in CE is sometimes recommended,[2,5,18,19] but such supplementation can have adverse health effects, and has not been extensively studied.[20,21]

We hypothesized that dogs with CE could have decreased concentrations of vitamin D and A. Our aims were (1) to determine whether serum FSV concentrations in dogs with CE differ from those in healthy dogs and (2) to identify correlations with sex, age at diagnosis, clinical signs, disease activity indexes and clinicopathological variables. Knowledge of serum concentrations of FSVs in dogs with CE may help establish guidelines for their clinical measurement and ultimately guide evidence-based recommendations for supplementation in dogs with vitamin deficiency.

2 | MATERIALS AND METHODS

2.1 | Animals, sample collection, and screening assays

Blood samples were prospectively collected from dogs presented to the University of Tennessee Veterinary Medical Center from June 2018 to December 2018 for investigation of CE for the measurement of FSV on the day of inclusion. Dogs were eligible for inclusion in the study if they had a clinical diagnosis of CE and were presented for chronic gastrointestinal signs lasting at least 3 weeks. To be enrolled in the study, records had to show fecal screening (fecal flotation) or deworming or both, as well as CBC and serum biochemistry profiles performed within 6 months before inclusion. At the discretion of the attending clinician, canine pancreatic lipase immunoreactivity (cPLI), canine trypsin-like immunoreactivity (cTLI), cobalamin, folate, and baseline cortisol concentrations also were measured. Additionally, dogs had to have abdominal imaging (ultrasonography or computed tomography) at the time of diagnosis. Dogs with evidence of abdominal or intestinal masses or any other indications of nongastrointestinal disease on abdominal imaging were excluded. All dogs included had urinalysis performed to rule out proteinuria, and hypoalbuminemic patients (defined as serum albumin concentration <2.5 g/dL) were required to have a urine protein: creatinine ratio <2 for inclusion to rule out clinically relevant glomerular proteinuria as a cause of the hypoalbuminemia.

All dogs presented with any of the following concomitant diseases known to affect FSV concentrations were excluded: protein-losing nephropathy and chronic kidney disease,[22-24] systemic neoplasia or ongoing chemotherapy,[25] critically ill dogs (including those with uncontrolled diabetes mellitus or acute pancreatitis),[26] exocrine pancreatic insufficiency,[27] idiopathic hypercalcemia or hyperparathyroidism.[28] Also, dogs were excluded if they were exclusively being fed a homemade diet or receiving calcium or vitamin supplementation.

For each dog, signalment, weight, body condition score (BCS), frequency, and type of clinical signs, diet, canine inflammatory bowel disease activity index (CIBDAI) and canine chronic enteropathy clinical activity index (CCECAI) were recorded. Additionally, results of infectious disease testing (*Histoplasma* antigen, MiraVista Labs, Indianapolis, IN) and histopathology reports from intestinal biopsy samples were documented when performed. As part of the control group, healthy client-owned adult dogs and healthy laboratory research dogs were recruited from clients and staff of the University of Tennessee Veterinary Medical Center and from the purpose-bred research colony dogs of the University of Tennessee College of Veterinary Medicine, respectively. Healthy dogs were selected based on the absence of any clinical signs of CE, having blood test results (CBC and serum biochemistry) within reference range, and negative fecal screening or deworming or both within 6 months before inclusion. Every dog enrolled in the study was fed a diet consistent with the recommendations of the Association of American Feed Control Officials for meeting vitamin requirements.

Blood samples were collected from all dogs by venipuncture after an 8-12 hour fast. Collection and storage of samples for FSV measurement followed guidelines established by the Michigan State University Veterinary Diagnostic Laboratory. Samples were collected into sterile blood collection tubes and placed immediately into light-protected containers. Samples then were centrifuged at 3500*g* for 10 minutes, and serum aliquoted into at least 3 aliquots and stored at −80°C until analysis. During sample handling, light exposure was minimized to decrease the risk of vitamin denaturation. Complete blood count, serum biochemistry, and serum cortisol concentration were performed by an

accredited veterinary diagnostic laboratory (University of Tennessee Veterinary Diagnostic Laboratory Service; Knoxville, TN). Analysis of cPLI, cTLI, cobalamin, and folate was performed by a diagnostic laboratory (Texas A&M Gastrointestinal Laboratory, College Station, TX) and the reference intervals provided were used for analysis.

2.2 | Vitamin analysis

All serum samples were stored at −80°C and the aliquots for FSV analysis were shipped on dry ice in a light-protected container to a commercial laboratory within 3 months of collection (Michigan State University Veterinary Diagnostic Laboratory, Lansing, MI).

Retinol and α-tocopherol were analyzed by isocratic ultraperformance liquid chromatography (UPLC). Samples were thawed upon arrival, and 500 μL was pipetted into a tube for extraction along with controls. In-house bovine and equine serum were used as controls. One milliliter total volume was obtained by addition of 0.9% sodium chloride. Internal standard, ethanol with butylated hydroxytoluene (BHT) and hexane were added. Samples were vortexed, centrifuged and an aliquot of the hexane layer was used to obtain extracts that then were dissolved in the UPLC mobile phase. Samples then were analyzed using a Waters Acquity system along with the Waters Empower Pro Chromatography Manager Software. Ultraviolet detection was used to obtain peaks at 325 nm for retinol and 292 nm for α-tocopherol, which then were manually reviewed after autointegration. Standards were run for both retinol and α-tocopherol and a 5-point calibration curve was constructed. Stock α-tocopherol was prepared by addition of α-tocopherol standard to the chromatographic mobile phase. The retinol solution was prepared the same way by addition of retinol standard to the chromatographic mobile phase. Reference ranges were provided by the laboratory for retinol and α-tocopherol as 400-1200 ng/mL and 4.00-12.00 μg/mL, respectively.

Analysis of 25(OH)D was performed at the Michigan State Veterinary Diagnostic Laboratory using a validated, commercially available radioimmunoassay kit (25-hydroxyvitamin D 125I RIA Kit, DiaSorin, Stillwater, MN) according to the manufacturer's recommendations. The reference range provided by the laboratory was 109-423 nmol/L. Multiple studies have shown the stability of vitamin D, retinol, and α-tocopherol under the aforementioned conditions of processing, storage and shipping.[29-31]

All sampling methods and experimental procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee (IACUC number #2590-0318), and signed written consent was obtained from every owner.

2.3 | Statistical analysis

The normality of the FSV concentrations was tested using the Shapiro-Wilk test. The retinol concentration was not normally distributed, and a nonparametric Mann-Whitney *U* test was used for comparison of FSV concentrations in dogs with CE to healthy dogs.

The correlations among retinol, α-tocopherol, and 25(OH)D concentrations and clinicopathological variables were calculated using Spearman's rank-order correlation test. Results were considered significant if $P < .05$.

Multiple regression analysis (SAS Proc Reg) was performed. The best adjusted squared, Akaike information criterion (AIC) and the sum of squared errors (SSE) method were used to select the best set of variables and the backward selection method was used to remove nonsignificant variables and retain significant variables. The dependent variables were retinol, α-tocopherol, and 25(OH)D concentrations and the independent variables were age, sex, appetite, presence or absence of vomiting and diarrhea, fecal score, BCS, CIBDAI, CCECAI, hematocrit, absolute neutrophil count, absolute lymphocyte count, absolute eosinophil count, absolute platelet count, and serum creatinine, BUN, albumin, globulin, cholesterol, total calcium, magnesium, cobalamin, and folate concentrations. Appetite was categorized as 1 = normal appetite, 2 = slightly decreased, 3 = hyporexia, and 4 = anorexia. The 25(OH)D concentrations were natural log transformed to meet the constant variance assumption. Three outliers in α-tocopherol and retinol were removed to meet the normality assumption. The variable with the highest P-value was removed until all remaining variables had a $P < .05$. A Shapiro-Wilk W and QQ normality plots were used to evaluate normality of the residuals. All statistical assumptions regarding linearity, normality, and constant variance were met.

For additional comparisons, CE dogs were divided into a low 25(OH)D group (defined as 25(OH)D < 109 nmol/L) and normal 25(OH)D concentration group (defined as 109 nmol/L < 25(OH)D < 423 nmol/L). Kruskal Wallis analysis was used to analyze 25(OH)D subgroups using all significant predictors selected from regression analysis using SAS 9.4. Least square mean was computed and separated by Fisher's least square different (LSD) methods. All analyses were analyzed using SAS Version 9.4, TS1M6 (SAS 9.4, Cary, NC: SAS Institute Inc; 2014). $P < .05$ was considered significant.

3 | RESULTS

Eighteen dogs with CE and 33 healthy controls were included in the study. In the CE group, the median age (range) was 7 years (0.7-13 years) and the median body weight (range) was 30 kg (3.7-39.3 kg). Sex distribution was as follows: 1 intact male, 8 castrated males, 1 intact female, and 8 spayed females. Dogs included in the study were of the following breeds: mixed breed (n = 4), English Springer Spaniel (n = 2), and 1 dog each of Yorkshire terrier, Portuguese water dog, Standard poodle, English Bulldog, German shepherd, Welsh Corgi, Shih Tzu, Jack Russell Terrier, Dachshund, Maltese, Cocker Spaniel, and Great Dane. The control group included 33 dogs: 5 client-owned dogs and 28 healthy purpose-bred laboratory dogs. The median age (range) was 2.0 years (2-7 years), and the median body weight (range) was 11.5 kg (2.3-30.0 kg). This group consisted of 13 castrated males and 20 spayed females of the following breeds: Beagle (n = 21), Red tick Coonhound (n = 7), mixed breed (n = 4), and Chihuahua (n = 1).

In the CE group, 6 dogs (33%) were fed a vegetarian commercial hydrolyzed diet, 4 dogs (22%) a commercial gastrointestinal support diet, 3 dogs (17%) commercial hydrolyzed diets, 3 dogs (17%) a mixture of type of commercial foods, 1 dog (5.5%) an adult maintenance commercial diet and 1 dog (5.5%) a mixed adult maintenance commercial and home-cooked diet. Thirteen dogs (72%) had unchanged appetite at the time of presentation, whereas 4 dogs (23%) were hyporexic. None of the dogs included were anorexic. Seven dogs (39%) were underconditioned with a BCS <5, 7 dogs (39%) were of normal weight with a BCS between 5 and 6, and 4 dogs (22%) were overweight with a BCS >6. Fifteen dogs (83%) were reported to have diarrhea at the time of presentation. Of those, 8 dogs (53%) had a fecal score between 4 and 5, and 7 dogs (47%) had a fecal score between 6 and 7.

Thirteen dogs (72%) were reported to be vomiting, including 7 (54%) dogs having >2 episodes per week and 6 dogs (46%) with a frequency of <1 episode per week.

Both the CIBDAI and the CCECAI were recorded in all 18 (100%) dogs. Eight dogs (44%) had a CIBDAI index between 0 and 3, 7 dogs (39%) between 4 and 5, 2 dogs (11%) as 7 and 1 dog (6%) as 12. Six dogs (33%) were scored with a CCECAI between 0 and 3, 1 dog (6%) between 4 and 5, 7 dogs (39%) between 6 and 8, 2 dogs (11%) had a CCECAI of 9 and 2 dogs (11%) of 12. Cobalamin and folate concentrations were measured in all but 1 dog with CE. Five of 17 (29%) had hypocobalaminemia. Three dogs (17%) in the CE group had low folate concentration, 2 of them had hypocobalaminemia as well. Sixteen of 18 dogs had cTLI measured, whereas only 11 of 18 had cPLI evaluated. None of the dogs had a cTLI below the reference range, but 3 of 18 (17%) had cTLI above the reference interval. Four dogs of 11 (36%) had cPLI concentrations above the reference range.

All 18 dogs had an ultrasound evaluation within 6 months before inclusion and had findings consistent with CE. Changes in wall thickness of the duodenum and jejunum, alterations in mucosal echogenicity with the presence of hyperechoic speckles, hyperechoic striation, or both, with or without mild increased in lymph node size were considered abdominal sonographic alterations that could be consistent with but not specific for CE.[32,33] Nine of 18 dogs (50%) had histologic evaluation of intestinal samples, including lymphoplasmacytic or eosinophilic enteritis (n = 4), or both, intestinal lymphangiectasia (n = 2), focal lipogranulomatous lymphangitis (1), and inconclusive (n = 2; Supplemental information S1).

Descriptive statistics on serum concentrations of retinol, 25(OH)D, and α-tocopherol measured in dogs in CE and control groups are reported in Table 1. Significant differences in serum retinol and serum 25(OH)D concentrations were found between the CE group and control group. Consistent with a previous study,[12] 25(OH)D concentrations were decreased in patients with CE compared with controls. Surprisingly, serum retinol concentrations were increased in dogs with CE compared with healthy dogs. None of the dogs with CE had retinol concentrations lower than those of the healthy dogs. Although serum α-tocopherol was not significantly different in dogs with CE compared to healthy dogs, a higher variability and wider spread of serum vitamin E concentrations were found among dogs with CE. Although all healthy dogs had α-tocopherol concentrations above the reference range, consistent with previous studies,[16,27] several dogs with CE had α-tocopherol concentrations within or even below the reference range. A panel of violin plots in Figure 1 illustrates the comparison of FSVs between the CE and healthy groups. Additional data on CBC and biochemistry results for individual patients are provided in Supplemental Information S2.

Significant correlations between clinical, clinicopathological variables and FSVs are listed in Table 2. Briefly, strong positive correlations were found between 25(OH)D concentration and calcium ($r_s = 0.7031$; $P = .001$) and albumin concentrations ($r_s = 0.6925$; $P = .002$). Also, a strong positive correlation was observed between serum α-tocopherol concentration and serum cholesterol concentration ($r_s = 0.7783$; $P = .0001$).

The final model of multiple regression analysis indicated that appetite score, presence of vomiting, fecal score, serum albumin concentration, and total eosinophil count were independent predictors of serum 25(OH)D concentration ($r_s = 0.7701$). Age, total neutrophil count, total platelet count, serum albumin concentration, serum globulin concentration, serum cholesterol concentration, and serum magnesium concentration were independent predictors of serum retinol concentration ($r_s = 0.7485$). Age, body weight, appetite, vomiting, diarrhea, BUN concentration, serum albumin concentration, serum cholesterol concentration, and serum calcium concentration were independent predictors of serum α-tocopherol concentration ($r_s = 0.7425$).

Subsequently, dogs with CE were divided into different subgroups based on 25(OH)D concentration (low and normal). All significant predictors selected from regression analysis were compared between the subgroups and control group. Dogs with low serum 25(OH)D concentrations had higher fecal scores ($P < .001$) and lower serum albumin concentrations ($P < .001$), as shown in Figure 2.

TABLE 1 Descriptive statistics and comparison among chronic enteropathy and control dogs for serum concentrations of retinol, 25(OH)D, and α-tocopherol.

	Reference range	n	Chronic enteropathy	n	Control group	*P* value
25(OH)D (nmol/L)	109-423	17	147 (11-387)	30	288 (133-468)	.01*
Retinol (ng/mL)	400-1200	18	757 (330-2274)	33	610 (281-906)	.03*
α-Tocopherol (μg/mL)	4.0-12.0	18	22.7 (3.3-73.5)	33	25.4 (14.2-53)	.87

Note: Data are reported as median and (range).
*Significant difference between groups. Significance set at $P < .05$.

4 | DISCUSSION

We investigated whether dogs with CE have lower concentrations of FSVs compared with healthy dogs. Consistent with previous literature, dogs with CE had lower 25(OH)D concentrations[11,13-16] compared to healthy dogs.[12] Although most dogs had retinol concentrations within the reference range, a few in both groups (CE and controls) had lower retinol concentrations. All healthy dogs had α-tocopherol concentrations above the normal reference range. Surprisingly, some dogs with CE included in this study had even higher concentrations of retinol and α-tocopherol than healthy dogs.

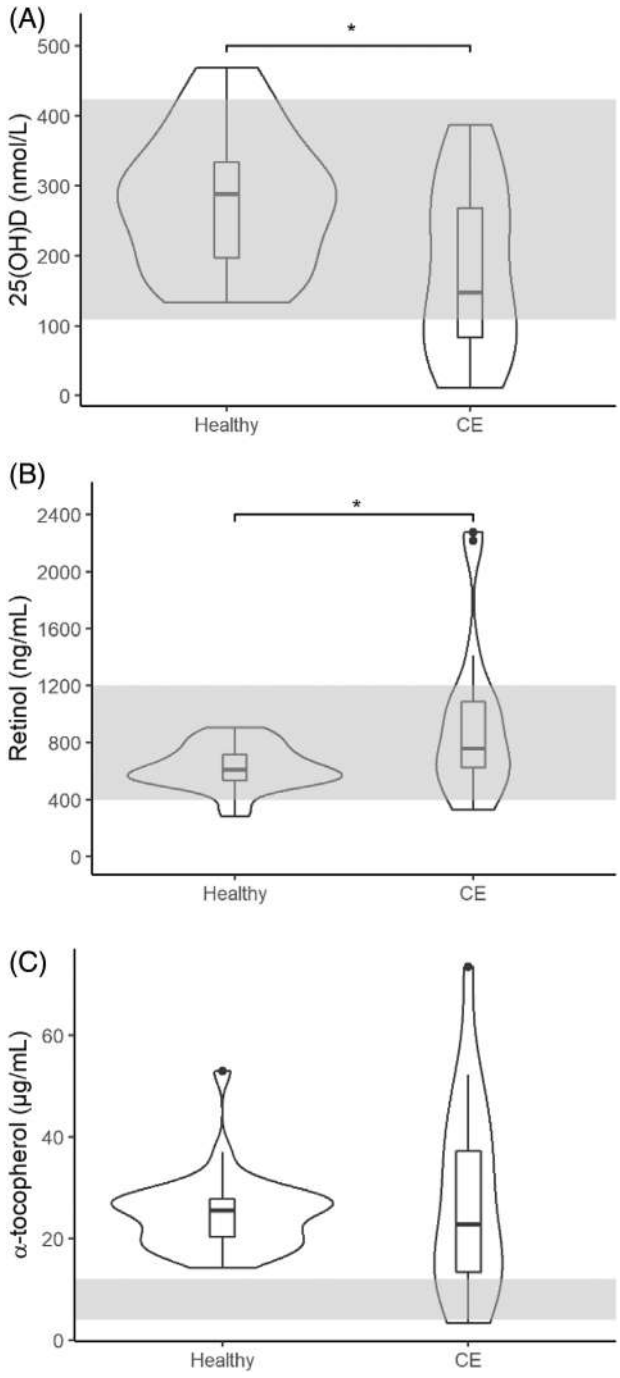

FIGURE 1 Violin plot showing the distribution of 25(OH)D, (A) retinol, (B) and α-tocopherol, (C) in healthy and CE dogs. The boxes represent the 25th and 75th percentiles and whiskers represent the interquartile range. The dots in B and C represent outliners. The star underlined that healthy and CE groups have a significantly different concentration of 25(OH)D and retinol ($P = .01$ and .03, respectively). α-tocopherol did not differ between the 2 groups ($P = .87$). Gray zone illustrates the reference range for the FSVs. CE, chronic enteropathy.

Vitamin D, measured as 25(OH)D, was significantly decreased in dogs with CE compared with healthy dogs. The median serum 25(OH)D concentration and (range) was 147 nmol/L (11-387 nmol/L), which is higher than the median value reported in other studies[11-16] and falls within the reference interval used by the designated laboratory (109-423 nmol/L). Only 6 of 17 dogs (35%) had serum 25(OH)D concentrations below the reference interval. The majority of these dogs were underweight (4/6), with a BCS <5, experienced vomiting (4/6), and had a fecal score between 6 and 7 (4/6), a CIBDAI between 5 and 12 (5/6), and a CCECAI between 8 and 12 (5/6). Three of 6 had hypocobalaminemia, and only 1 had concurrently low folate concentration. Also, we found that serum calcium and albumin concentrations were positively correlated with serum 25(OH)D concentrations in dogs with CE. Notably, the 6 dogs with low serum 25(OH)D concentrations also had serum total calcium and albumin concentrations below the reference interval. Unfortunately, ionized calcium measurements were not performed in most cases, and the clinical relevance of low calcium concentration and the impact of hypoalbuminemia is unclear. Previous studies have linked low ionized calcium concentration with hypovitaminosis D, hypomagnesemia and low PTH concentration in dogs with CE.[12,34,35] Most vitamin D metabolites circulate in a protein-bound form, with albumin and vitamin D binding proteins binding >99% of these circulating vitamin D metabolites. Although our study did not measure vitamin D binding protein, a recent study showed that serum vitamin D binding protein concentrations were similar between dogs with low and normal 25(OH)D concentrations. However, serum albumin concentrations were significantly lower in dogs with low 25(OH)D concentrations.[16] These findings support an underlying pathophysiology leading to decreased 25(OH)D and albumin, potentially caused by inadequate intake provided by the diet, lymphatic dysfunction, alterations in intestinal permeability and absorption, or as a marker of inflammation. In the future, measurement of free vitamin D concentrations could provide additional insight into the mechanism of vitamin D deficiency. Currently, the decreased vitamin D concentrations are likely multifactorial but could contribute to the pathogenesis of CE, considering the anti-inflammatory and immunomodulatory actions of vitamin D.[36]

We also measured vitamin A concentrations as retinol. Retinol is a late indicator of vitamin A deficiency and does not decrease until vitamin A deficiency is extremely severe.[37] Thus, mild vitamin A deficiency cannot be ruled out in our patients. Interestingly, we found that dogs with CE had increased retinol concentrations compared with healthy controls. A previous study reported increased retinol concentrations in dogs with CE and low 25(OH)D concentrations, even higher than our results.[16] However, retinol is likely to be a very insensitive marker of total body vitamin A status. In fact, the majority of vitamin A in dogs and cats circulates as retinyl esters, which are more sensitive markers of vitamin A deficiency.[38,39]

TABLE 2 Correlation between retinol, α-tocopherol and 25(OH)D concentration and clinical pathological variables.

Variable		Spearman's correlation score	*P* value*
Retinol	α-Tocopherol	0.64	.004
	Albumin	0.57	.01
	Cholesterol	0.57	.01
	Calcium	0.54	.02
	Folate	0.53	.03
25(OH)D	Calcium	0.70	.001
	BUN	0.56	.02
	Albumin	0.69	.002
	Cholesterol	0.56	.02
	Creatinine	0.49	.05
	Globulin	0.49	.04
	Neutrophils	−0.51	.04
	CCECAI score	−0.49	.05
α-Tocopherol	Cholesterol	0.78	<.001
	Retinol	0.64	.004
	Cobalamin	0.63	.01

Abbreviation: CCECAI, canine chronic enteropathy activity index.
*P-value as assessed by Spearman correlation. Significance set at $P < .05$.

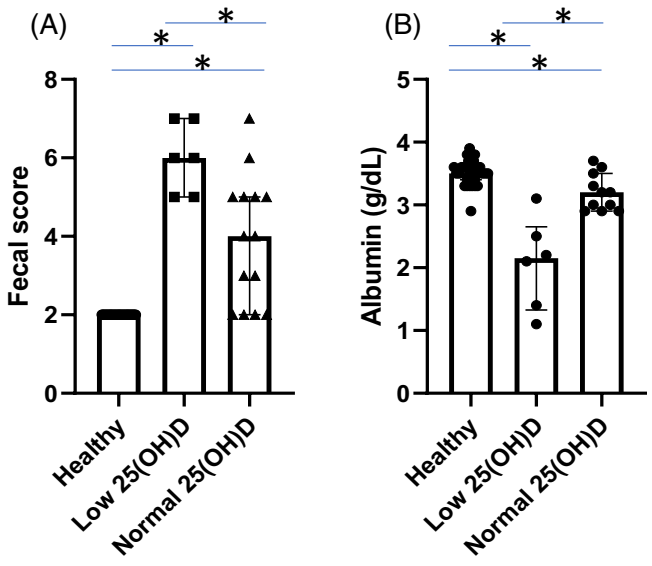

FIGURE 2 Graphic representation of serum albumin, and fecal score in dogs with different concentrations of 25(OH)D and healthy dogs. The boxes represent the 25th and 75th percentiles and whiskers represent the interquartile range. The star underlined significance set as $P < .5$.

In our study, the multiple regression model for retinol included possible markers of inflammation (neutrophil and platelet counts, serum globulin concentration, and albumin concentration) and malabsorption (serum cholesterol, albumin, and magnesium concentrations). In fact, in humans, serum retinol concentration is influenced by retinol-binding protein, inflammatory and nutritional status, as well as the concentrations of other nutrients such as zinc and magnesium.[40,41]

Although serum α-tocopherol concentrations were not significantly different in dogs with CE compared with healthy dogs, the dogs with CE exhibited higher variability and a wider spread of serum vitamin E concentrations, suggesting potential alterations in vitamin E availability, metabolism, or transport. In our study, healthy dogs as well as dogs with CE had α-tocopherol concentrations above the reference range reported by the laboratory, and only a few dogs with CE had concentrations below or within the reference range. Although the clinical relevance of this finding is unknown, our results are consistent with 2 other studies reporting α-tocopherol concentrations above the reference interval in dogs with CE[16] and those with exocrine pancreatic insufficiency.[27] The authors of that study questioned the need of new reference intervals for dogs fed commercial diets, considering the vitamin E concentrations in standard commercial dog foods. These results emphasize the importance of including a control group in such studies.[16,27] The only dog with α-tocopherol concentration below the reference range was severely affected by CE. This dog was underconditioned and had diarrhea with a fecal score of 7/7. Laboratory testing showed hypoalbuminemia, hypocalcemia, hypocholesterolemia, hypocobalaminemia, and hypovitaminosis D. Unfortunately, an intestinal biopsy was not performed. These findings are in agreement with the results of our multiple regression analysis. Moreover, an association between α-tocopherol and serum cholesterol concentration was identified. Interestingly, recent studies have indicated that some cholesterol transporters can also transport vitamin E and vitamin K, and potentially influence intestinal absorption of these vitamins as well.[42] Also, the vitamin E/cholesterol ratio has been suggested to be more accurate determinant of vitamin E status, particularly in certain conditions such as cholestasis.[43]

Our study had some limitations. We included a limited number of cases and additional larger studies are needed. The lack of standardization of diet and treatment introduces another potential variable that could have influenced serum vitamin concentrations. Although we did not find a significant association between serum vitamin concentrations and diet, the influence of diet cannot be excluded. Also, some dogs were receiving glucocorticoids at the time of inclusion, and the influence of glucocorticoids on vitamin D metabolism is controversial.[44,45] However, our goal was to document FSV concentrations in dogs with CE in a clinically relevant setting (eating commercial diets and receiving appropriate treatment) to describe potential deficiencies and identify the possible need for supplementation. Also, not all patients had an intestinal endoscopy performed, and in the histopathology reports the World Small Animal Veterinary Association (WSAVA) gastrointestinal score was missing. Our results suggest that future research should focus on the evaluation or supplementation of FSVs in dogs with severe CE or protein-losing enteropathy. Furthermore, our control group included mostly young Beagles from a colony, that all were fed a standard diet and lived in similar conditions compared with the highly variable case group.

In conclusion, dogs with CE and severe clinical signs such as decreased appetite, vomiting, poor body condition, poor fecal scores, and low serum calcium and albumin concentrations should be monitored for decreased 25(OH)D concentrations. Additional studies are needed to understand the benefits of supplementation or correction of FSV concentrations. The clinical relevance of the increased concentrations of retinol and α-tocopherol in dogs with CE remains unclear. This finding could represent alterations in vitamin A and E metabolism in dogs with CE, and investigating this aspect in dogs receiving standardized diets, with measurement of retinyl esters, should be considered in future studies.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the University of Tennessee IACUC, number 2590-0318, and signed written consent obtained from every owner.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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