

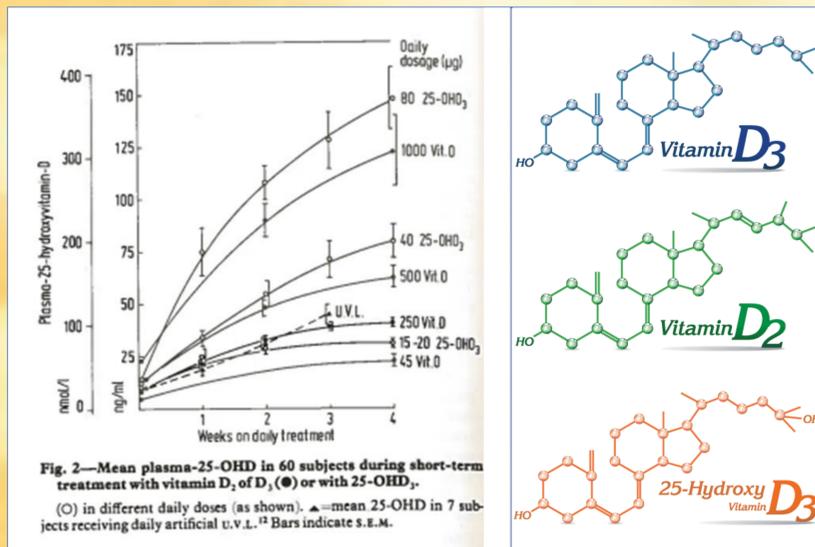
# Two trials to investigate techniques for canine Vitamin D supplementation

Tyler Senft, Robert Backus, Lauren Young

Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO

## Background

- Vitamin D deficiencies have been seen in canines diagnosed with lymphoma, hemangiosarcoma, mast cell neoplasia, and other non-cancerous diseases (Selting et. al. 2014)
- Vitamin D is obtained in canines exclusively through diet and supplementation
- The most common form of Vitamin D supplementation is Vitamin D3 given in an oil capsule; Vitamin D2 is often used in the medical community
- Anecdotal reports indicate that Vitamin D2 in oil may have low bioavailability in canines
- Human studies indicate that 25(OH)D3 is much more potent at increasing serum Vitamin D levels
- Vitamin D equilibration occurs significantly faster when supplementing with 25(OH)D3 compared to Vitamin D3



Stamp et. al. 1977. Comparison of oral 25-Hydroxycholecalciferol, Vitamin D, and ultraviolet light as determinants of circulating 25-Hydroxyvitamin D. *The Lancet*. 1341-1343. Compares the serum levels of 25(OH)D3 after supplementation with Vitamin D2, D3, and 25(OH)D3

## Objectives

### Trial 1

- Determine whether canine Vitamin D2 supplementation is more effective in raising Vitamin D status when given in an oil solution or an ethanol solution on a semi-moist dog treat

### Trial 2

- Determine if an ethanol-based 25(OH)D3 solution will maintain concentration on a semi-moist dog treat during storage

## Hypothesis

### Trial 1

- Vitamin D2 given in an ethanol solution on a treat will more effectively raise serum 25(OH)D2 levels than in an oil solution

### Trial 2

- 25(OH)D3 will maintain a stable concentration on semi-moist dog treats over the period tested

## Materials and Methods

### Trial 1

- Animals: 2 purpose-bred Adult Male Healthy Chinese Crested-Beagle crosses (10.2 kg and 9.7 kg)
- Each dog was supplemented with a 2.3  $\mu\text{g}/\text{kg}$  of  $\text{BW}^{0.75}$  dose of Vitamin D2 once daily in either an olive oil solution on food or an ethanol solution on a semi-moist dog treat with food. Serum levels of 25(OH)D2 were analyzed using HPLC on Day 0, Day 8, Day 14, and Day 21 of Vitamin D2 supplementation

### Trial 2

- 20  $\mu\text{l}$  of a 200  $\mu\text{g}/\text{ml}$  (2ug) ethanol solution of 25(OH)D3 were placed on semi-moist dog treats weighing 4.0-5.4 g each
- Half of the treats were placed in refrigeration (4° C), and the other half were placed in a room temperature (23° C) cabinet. Both groups were stored in Ziploc bags placed within amber bags
- 25(OH)D3 concentration was measured on Day 0, Day 7, Day 14, and Day 21 of storage for both groups using reverse and normal phase HPLC

## Results

### Trial 1: Vitamin D2 Supplementation Oil vs. Ethanol

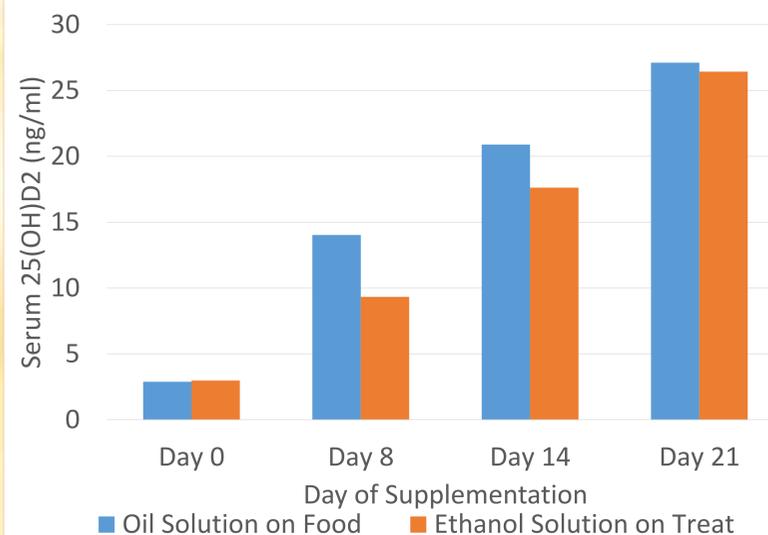


Figure 1: Serum 25(OH)D2 concentration in Beagle-Chinese Crested Crosses over time when given daily supplementation of 2.3  $\mu\text{g}/\text{kg}$  of  $\text{BW}^{0.75}$ . Blue bar represents canine that was given Vitamin D2 supplement in oil, and orange bar represents canine that was given Vitamin D2 supplement in ethanol on a treat

### Trial 2: 25(OH)D3 Stability in Refrigeration vs. Room Temperature

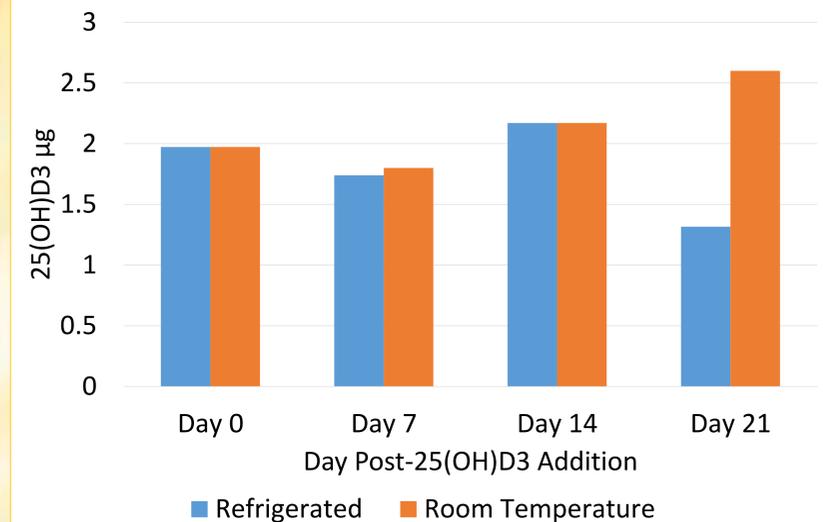


Figure 2: Concentration of 25(OH)D3 on semi-moist dog treats over the period of time tested. 2  $\mu\text{g}$  of 25(OH)D3 in an ethanol solution were added to the treats on Day 0 and measured each week

## Conclusions and Discussion

### Trial 1

- Vitamin D2 given in an oil solution with food appeared to be more effective at raising serum 25(OH)D2 concentration
- Vitamin D2 given in an oil solution leads to equilibration faster than Vitamin D2 given in an ethanol solution
- This is consistent with what has been found in humans
- Both Vitamin D2 in an oil solution and Vitamin D2 in an ethanol solution reach a similar level of equilibration

### Trial 2

- 25(OH)D3 concentration remained stable on semi-moist dog treats during 14 days in refrigeration and 21 days in room temperature
- Room temperature may be a more suitable storage temperature to prolong 25(OH)D3 on dog treats
- This may be applicable to future clinical trials

## Acknowledgements

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