Two trials to investigate techniques for canine Vitamin D supplementation

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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

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 µg/kg of BW of 25(OH)D3 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 µl of a 200 µg/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**

![Graph showing serum 25(OH)D2 concentration comparison between oil and ethanol solutions](image)

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

![Graph showing concentration of 25(OH)D3 over time in refrigeration and room temperature](image)

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

Funding for this project was provided by the Nestle Purina Small Animal Endowment Fund. A special thanks to Dr. Kaoru Tsuruta and Bobby Backus for their support. Thank you to the Veterinary Research Scholars Program and the University of Missouri Department of Veterinary Medicine and Surgery for giving me this opportunity.