Original communication

Serum 25-hydroxyvitamin D Concentrations in Captive and Free-ranging, White-tailed Deer (*Odocoileus virginianus*)

W. Ray Waters[1], Brian J. Nonnecke[1], Samantha E. J. Gibbs[2], Michael J. Yabsley[2], Stephen M. Schmitt[3], Melinda K. Cosgrove[3], Mitchell V. Palmer[1], Tyler C. Thacker[1], Steven C. Olsen[1], Ronald L. Horst[1] and Timothy A. Reinhardt[1]

[1]National Animal Disease Center, Ames, Iowa, USA
[2]Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA
[3]Michigan Department of Natural Resources, Rose Lake Wildlife Disease Laboratory, East Lansing, Michigan, USA

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Abstract: Serum concentrations of 25-hydroxyvitamin D [25(OH)D] were determined for free-ranging and captive white-tailed deer (WTD). Effects of gender, season, and age on 25(OH)D concentrations were determined as well as comparisons to concentrations in serum from captive reindeer and elk. Seasonal variations in 25(OH)D concentrations were detected for both captive and free-ranging WTD with greatest concentrations detected in August/September (~ 25 ng/mL) and lowest concentrations in February (~ 5 – 10 ng/mL). Free-ranging WTD < 1 year of age had lower 25(OH)D concentrations (~ 6 ng/mL) than did free-ranging WTD > 1 year of age (~ 12 ng/mL). For captive WTD fawns, 25(OH)D concentrations increased from 1 to 9 days of age (exceeding 100 ng/mL) and then steadily declined to ~ 10 ng/mL by 3 months of age. In general, differences in 25(OH)D concentrations based on gender were not detected. 25(OH)D concentrations in captive WTD did not differ from that of captive reindeer; yet, 25(OH)D concentrations were lower in WTD than in captive elk. Additional research is necessary to determine if low serum 25(OH)D concentrations during the winter or pre-weaning period are associated with increased rates of infectious and metabolic disease.

Key words: Vitamin D, white-tailed deer, elk, reindeer, neonate, captive cervids, free-ranging cervids

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Introduction

Vitamin D is essential for calcium homeostasis, cell differentiation / proliferation, and innate as well as adaptive immune function. Deficiencies in Vitamin D are associated with increased susceptibility to infectious disease, osteomalacia, osteopenia, osteoporosis, muscle weakness, cancer, autoimmune disease, and cardiovascular disease [1]. Vitamin D is derived from two sources: dietary intake (vitamin D_2 from plant sources or D_3 from animal sources) and by the conversion of 7-dehydrocholesterol to cholecalciferol (i.e. pre-vitamin D_3) in the skin, a reaction catalyzed by ultraviolet light. At body temperature, cholecalciferol spontaneously converts to vitamin D_3. Vitamin D-binding protein aids in the transport of vitamin D from the skin to the liver, where it is converted to 25-hydroxyvitamin D [25(OH)D], the predominant circulating form. In response to hypocalcemic states, 25(OH)D is hydroxylated to 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D], the major mediator of biological activity for this vitamin.

In humans, "normal" circulating concentrations of 25(OH)D have traditionally been defined as 22–30 ng/mL and 1,25-$(OH)_2$D from 25–34 pg/mL [2]. Recently, it has been argued that these concentrations reflect "sun shy" cultural changes in our society and that normal ranges are more accurately defined by evaluation of individuals in "sun rich" cultures where clothing or cultural practices do not prevent sun exposure (i.e., 54–90 ng/mL for 25(OH)D) [3]. Evaluation of functional biomarkers such as circulating parathyroid hormone, calcium absorption, and bone mineral density in humans suggest that deficiency occurs when serum 25(OH)D concentrations fall below 32 ng/mL [3]. Deficiencies may result from inadequate dietary intake or sun exposure, breastfeeding (poor Vitamin D_3 content in human milk), aging, decreased bioavailability, increased catabolism, decreased synthesis (liver failure or chronic kidney disease), increased loss (nephritic syndrome), and obesity (sequestration of Vitamin D_3 in fat) [1]. Vitamin D status measurements and metabolism in domestic livestock are not significantly different from humans [4]. The "normal" observed range for circulating 25(OH)D in domestic livestock is 15–60 ng/mL [5]. Serum 1,25-$(OH)_2$D levels are more variable in domestic livestock than humans, with a range of 20->200 pg/mL, but the high values reflect the extreme calcium demands for egg and milk production [4,5]. Similar data on appropriate serum concentrations and causes of deficiency for many alternative livestock, captive wildlife, and free-ranging wildlife are not available. In particular, "normal" ranges for serum vitamin D metabolites of free-ranging and captive white-tailed deer (WTD, *Odocoileus virginianus*) have not been reported.

Anecdotal and epidemiologic studies indicate that vitamin D therapy is beneficial in the treatment of tuberculosis and low serum levels of vitamin D are associated with increased susceptibility [6]. Studies with tuberculosis in cattle indicate that: (1) serum 1,25-$(OH)_2D_3$ increases in the first two weeks following infection and is detectable within tuberculous granulomas [7]; (2) 1,25-$(OH)_2D_3$ reduces antigen-specific proliferation and interferon-γ production yet enhances nitric oxide production [7,8]; and (3) 1,25-$(OH)_2D_3$ decreases antigen-induced proliferation and activation marker expression by CD4+ T cells responding to *Mycobacterium bovis* antigen [9]. Extension of similar studies to cervid species (especially WTD) would benefit from an initial survey of serum vitamin D metabolite concentrations, particularly as related to age, gender, and season for determination of rationale treatment (*in vitro* or *in vivo*) ranges.

Circulating 25-(OH)D concentrations are the only measure of an animal's vitamin D status and the objective of the present study was to evaluate/compare the vitamin D status of free-ranging and captive WTD. Effects of gender, season, and age were evaluated when applicable as well as comparisons to captive reindeer (*Rangifer tarandus*) and captive elk (*Cervus elaphus* subsp. *nelsoni*). The context of these studies is that vitamin D deficiency may result in increased susceptibility to metabolic and infectious disease (e.g., *Mycobacterium bovis* infection [10] observed in free-ranging WTD in Michigan [11] and Minnesota). Thus, it is critical to first determine the concentration of 25(OH)D in WTD, both captive and free-ranging, to define a target range for determining relevant biomarkers for immune deficiency.

Materials and Methods

Serum samples were from hunter-killed WTD in various locales in Michigan (latitude ~44° N), Alabama (latitude ~30–35° N), Georgia (latitude ~30–35° N), Louisiana (latitude ~29–32° N), and Virginia (latitude ~36–39° N). Assessment of browse intake for vitamin D metabolite concentrations from these various locales was not possible due to the wide variation in available browse, based on location and season. Serum was also obtained from captive WTD, reindeer, and elk housed at the National Animal Disease Center, Ames, Iowa (latitude 41° N) accor-

Table I: Group sizes based on season, gender, and age for determination of serum 25(OH)D concentrations.

	Southeastern Free-Ranging WTD	Iowa Captive Deer
Season		
February	17	18
May/June	27	10
August/September	18	8
November/December	62	8
Gender		
Male	44	nd
Female	80	nd
Age		
< 1 yr	11	nd
1–2 yrs	37	nd
2–3 yrs	25	nd
3–4 yrs	22	nd
4–5 yrs	13	nd
> 5 yrs	16	nd

nd = not determined. Additionally, 137 and 30 samples were evaluated from free-ranging Michigan WTD in February and April – August, respectively.

ding to institutional animal care guidelines. The diet of captive WTD, reindeer, and elk consisted of grass/alfalfa hay, grass forage, and a vitamin D_3-fortified (953 IU/kg) commercial diet (Kent Feeds, Trophy Image 20, Muscatine, IA) *ad libitum*. Due to the secretive and inconsistent eating habits of captive WTD and large herd size, an actual estimation of the quantity of ingestion of the vitamin D_3 fortified diet was not possible. Group sizes are provided in Table 1. For southeastern US free-ranging WTD, the age of animals at sample collection was approximated by evaluation of dentition (n = 124) by trained scientists/technicians. Ages of deer sampled in Michigan were not available. Serum 25-(OH)D and 1,25-$(OH)_2$D were quantified by radioimmunoassay (RIA) using the methods of Hollis *et al.* [12, 13]. Intra- and inter-assay coefficients of variation were 9.4 and 10.8 %, and 16.2 and 13.1 % for 25-(OH)D and 1,25-$(OH)_2$D, respectively. 1,25-(OH)2D assay were performed only on selected samples due to sample size limitations. Briefly, serum 25-(OH)D was determined as follows. The 25-(OH)D standards (0–240 ng/mL) were prepared in vitamin D-deficient calf serum. Then 50 uL of sample or standards were extracted in 500 uL of acetonitrile by vortexing for 10 minutes, followed by centrifugation for 10 minutes at 2000 x g. Samples and standard extracts (25 uL) were pipetted into 12x75 mm glass tubes with 10,000 counts per minute of iodinated 25-(OH)D (Diasorin, Stillwater, MN). To these tubes, 1mL of anti-25-(OH)D (Diasorin) , were added; the tubes were then vortexed and incubated at room temperature for 90 minutes. Next, 0.5 mL of secondary antibody (Diasorin) was added, and the tubes were vortexed and incubated for 20 minutes at room temperature. At the end of the incubation, 0.75 mL of buffer (50 mM monobasic sodium phosphate, 0.1 % gelatin, 0.1 % sodium azide, 0.025 % Tween 20, pH 6.2) was added to the tubes followed by centrifugation at 2000 x g for 10 minutes. The supernatant was discarded and the tubes were counted in a gamma counter. Serum 25-(OH)D concentrations were determined from the standard curve. The 1,25-(OH)D assay had similar, additional purification steps that are described in Hollis *et al.*, 1996 [12]. Serum samples for assays were stored at -20C without freeze/thaw cycles. For free-ranging WTD, samples were collected by hunters (MI), Department of Natural Resources personnel (MI), and scientists / technicians (southeastern US) at the time of killing.

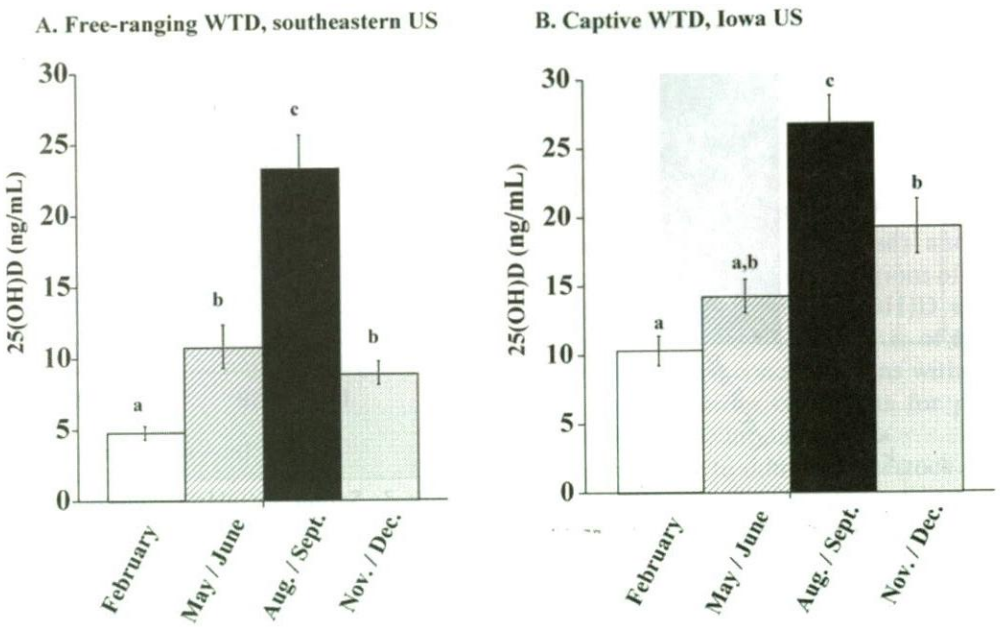

Figure 1: Effect of season on serum 25(OH)D concentrations in free-ranging, southeastern US (A) and captive, Iowa US (B) white-tailed deer (WTD). For graph A, n = 17, 27, 18, and 62 for February, May/June, August/September, and November/December, respectively. For graph B, n = 18, 10, 8, and 8 for February, May/June, August/September, and November/December, respectively.
Bars with differing letters (a – c) differ ($p<0.05$) within each graph.

Data were analyzed as a completely randomized design using Statview software (version 5.0, SAS Institute Inc, Cary, NC). Each animal served as the experimental unit in the analysis of all data. Effects of treatments on serum 25(OH)D concentration was analyzed using pooled data or as a split-plot with factorial ANOVA. Season, age (< 1 year of age vs. > 1 year of age), and cervid type (i.e. white-tailed deer, elk, and reindeer) were included as fixed effects, and animal was included as the random effect. Fisher's protected-LSD was applied when effects ($p<0.05$) were detected.

Results

Serum 25(OH)D concentrations did not differ ($p>0.05$) between Alabama, Georgia, Virginia, and Louisiana; thus, the data were consolidated for these four states and results presented as southeastern US. Effects of season on serum 25(OH)D concentrations were significant ($p<0.05$) in captive WTD in Iowa and free-ranging WTD in the southeastern US (Figure 1). Concentrations were greatest during August/September (~25 ng/mL) and lowest in February (~5–10 ng/mL). Similarly, 25(OH)D concentrations were lower ($p< 0.01$) in samples collected from Michigan WTD in February (14.2 ng/mL ± 1.0, n = 137) as compared to those obtained from April to August (24.9 ng/mL ± 2.2, n = 30).

Free-ranging WTD < 1 year of age had lower 25(OH)D concentrations (~6 ng/mL) as compared to deer > 1 year of age (~12 ng/mL) (Figure 2). Further analysis by individual year revealed that free-ranging WTD <1 year of age (n = 11) had lower ($p<0.05$) 25(OH)D concentrations than did deer 1–2 years of age (n = 37), 2–3 years of age (n = 25), or > 5 years of age (n = 16). Serum 25(OH)D concentrations did not differ ($p>0.05$) between age groups for free-ranging WTD in each of the age groups from > 1 to > 5 years of age regardless of season (1–2 years = 12.2 ng/mL, 2–3 years = 11.7 ng/mL, 3–4 years = 9.7 ng/mL, 4–5 years = 10.5, and > 5 years = 11.8 ng/mL). Seasonal effects were significant ($p<0.05$) within each of the age groups (i.e. by year of age) except for free-ranging WTD > 5 years of age.

To evaluate 25(OH)D concentrations during the neonatal period, samples were collected from captive WTD in Iowa periodically from 1–125 days of age (Figure 3). Fawns were retrieved from the breeding herd pasture at ~1 day of age after natural ingestion of colostrum (successful transfer confirmed by antibody

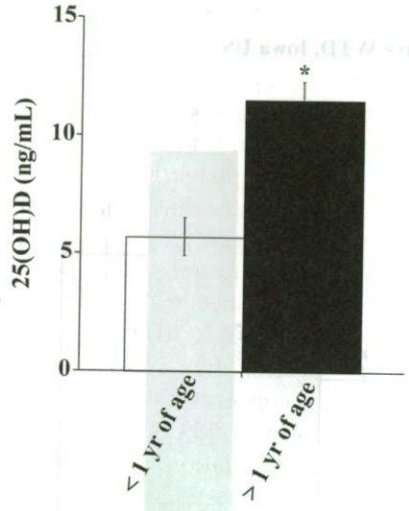

Figure 2: Age variation in serum 25(OH)D levels in free-ranging white-tailed deer (WTD) from the southeastern US.
* Serum 25(OH)D concentrations in WTD > 1 year of age (n = 113) were greater ($p<0.05$) than those in WTD < 1 year (n = 11).

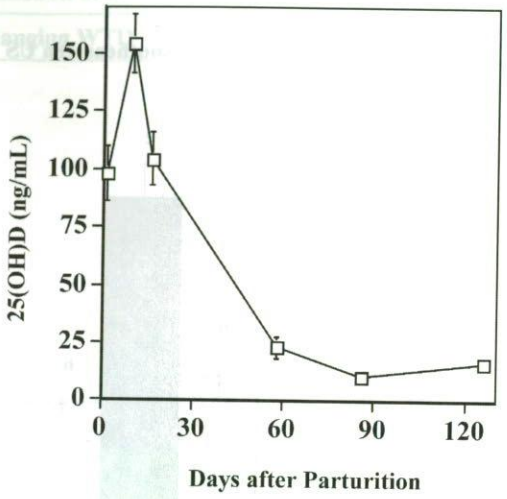

Figure 3: Serum 25(OH)D concentrations in newborn fawns from captive white-tailed deer (WTD) in Iowa, US. Newborn fawns (n = 7) received colostrum from their dams and were subsequently moved into a low-containment animal holding facility where they received goat's milk until weaning at ~ 60 days of age. Fawns also received grass hay and a commercial cervid diet *ad libitum*. Similar findings were observed with fawns from a previous year (n = 12) that also received goat colostrum from 1–7 days of age.

analysis, data not shown) and moved into a low-containment animal holding facility. Serum 25(OH)D concentrations increased ($p<0.05$) from 1 to 9 days of age to concentrations exceeding 100 ng/mL and subsequently declined ($p<0.05$) until 90 days of age. Animals were not exposed to natural sunlight during this time period (i.e. 1–90 days of age) and their diet consisted of goat's milk until weaning at ~60 days of age. Fawns also received *ad libitum* grass hay and a commercial cervid diet (Kent Feeds, Trophy Image 20) providing 953 IU of 25(OH)D/kg of feed. Feed intakes were not recorded.

Gender-specific differences were not detected (males, n = 44; females, n = 80) in free-ranging WTD except when the interaction of gender and season were considered. Serum 25(OH)D concentrations were lower ($p<0.05$) in samples collected in August/September from male free-ranging southeastern US WTD (17.9 ± 1.8, n = 3) as compared to female WTD (24.4 ± 2.7, n = 15). Differences based on gender at other sampling time-points (i.e. February, May/June, and November/December) were not evident.

Serum 25(OH)D concentrations in captive WTD were also compared to concentrations in sera obtained from captive elk and reindeer. All animals were of similar age (~ 1–3 years), fed the same ration, housed in adjacent paddocks, and sampled in June. Serum 25(OH)D concentrations in WTD (~ 14 ng/mL, n = 10) did not differ from that of reindeer (~ 18 ng/mL, n = 18); yet, 25(OH)D concentrations were lower ($p<0.05$) in WTD than in elk (~40 ng/mL, n = 19) (Figure 4).

The active form of the vitamin ($1,25(OH)_2D$) was also evaluated in a select set of samples. For free-ranging southeastern US WTD, serum $1,25(OH)_2D$ concentrations were lower ($p<0.05$) in May/June samples (13.9 pg/mL ± 1.4, n = 17) as compared to November/December samples (25.2 pg/mL ± 1.9, n = 29). Serum $1,25(OH)_2D$ concentrations in samples collected in September from Iowa captive WTD averaged 26.7 pg/mL ± 2.8 (n = 20) and ranged from 7.1 to 57.3 pg/mL. Samples collected at other time points from captive and free-ranging deer were not evaluated for $1,25(OH)_2D$.

Discussion

Despite supplementation with vitamin D_3-fortified diets, seasonal fluctuations in 25(OH)D concentrations in captive WTD followed a similar pattern to that of free-ranging WTD (i.e. unsupplemented) and 25(OH)D concentrations did not differ dramatically between captive and free-ranging WTD. Vitamin D deficiency is common among normal breastfed human infants, particularly during winter months [14]. Likewise, current findings indicate that free-

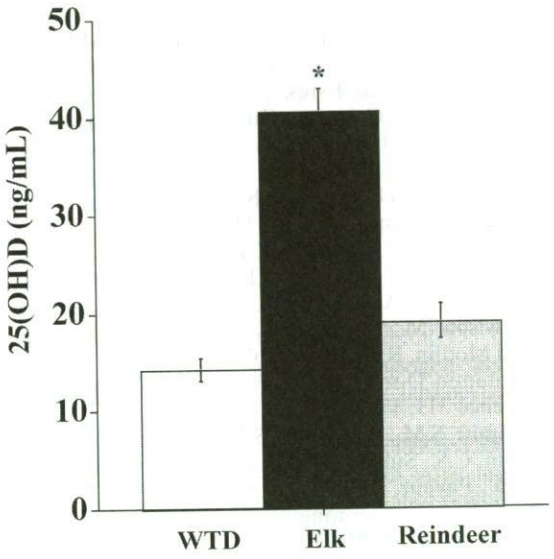

Figure 4: Comparison of serum 25(OH)D concentrations in captive white-tailed deer (WTD), elk, and reindeer in Iowa, US. WTD (n = 10), elk (n = 18), and reindeer (n = 19) were of similar age (~ 1–3 years), fed the same ration, housed in adjacent paddocks, and sampled in June.
* 25(OH)D concentrations in serum from elk exceed ($p<0.05$) those in WTD and reindeer.

ranging WTD less than 1 year of age have lower 25(OH)D concentrations than do older free-ranging WTD and concentrations are lowest in winter months for both free-ranging and captive WTD. Unexpectedly, neonatal captive WTD had transiently high serum 25(OH)D concentrations exceeding 100 ng/mL within 2 days of birth, which precipitously dropped to ~10 ng/mL by 3 months of age. The source of serum 25(OH)D in 1–9 day old fawns was not clear; however, it may be from colostral or *in utero* transfer.

In contrast to 25(OH)D, $1,25(OH)_2D$ concentrations were lower in May/June samples from free-ranging WTD as compared to November/December samples. These findings are consistent with prior studies with Roe deer demonstrating a spike in $1,25(OH)_2D$ concentrations from December through February [15]. The large variation in serum $1,25(OH)_2D$ concentrations (range 7.1 to 57.3 pg/mL) in the current study is consistent with the variation detected by Sempere *et al.*, [15] for Roe deer (range ~1 to 100 pg/mL). With the Roe deer study, $1,25(OH)_2D$ concentrations positively correlated with antler length and were associated with extra-renal production by antler cells. In the current study, differences in $1,25(OH)_2D$ concentrations between males and females were not detected; however, others have demonstrated that $1,25(OH)_2D$ serum concentrations increase during the period of antler mineralization in WTD [16] and fallow deer [17]. In contrast, antler growth does not affect 25(OH)D concentrations [15,17].

While critical for calcium homeostasis, Vitamin D is also an important immune modulator affecting B cell, T cell, and macrophage responses [10,18–21]. Recent evidence indicates that vitamin D-associated antimicrobial pathways differ between species (i.e. human as compared to mouse) and become inefficient when serum concentrations of 25(OH)D fall below ~ 30 ng/mL [10]. 25(OH)D concentrations vary between species; thus, it is of importance to determine *in vivo* concentrations within hosts of interest under various conditions for potential relevance to immune modulation.

As compared to domestic livestock [5], 25(OH)D concentrations in WTD and reindeer were similar to that reported for turkey and sheep, yet lower than that observed for chicken, cattle, and pigs. Except for adult chickens, serum $1,25(OH)_2D$ concentrations in WTD were lower than that reported for domestic livestock [5]. For free-ranging WTD, the quality of the sample varied with samples from Michigan, potentially affecting the concentration of 25(OH)D detected. For this reason, we did not directly compare results from Michigan WTD to results from the southeastern US WTD. With that said, 25(OH)D concentrations from Michigan WTD were within an expected range. Limitations on efficient sample collection from free-ranging WTD, at least with certain scenarios, are largely unavoidable. However, as samples were collected similarly throughout the study, the impact of sample quality on parameters such as age, gender, and season should be minimal.

Preliminary comparisons between WTD, elk, and reindeer indicate that 25(OH)D concentrations in serum from elk exceeded that of WTD and reindeer. As compared to WTD and reindeer, elk have noticeably robust antibody and weak cellular immune responses to brucellar and mycobacterial antigens [22,23]. Antibody-promoting actions of vitamin D are most likely via enhancement of T helper 2 responses and inhibition of T helper 1 responses [18,24,25]. Indeed, administration of vitamin D as an adjuvant with vaccines elicits Type 2 cytokine/antibody isotype profiles, shifting the response away from a Type 1 profile [19,26]. With humans, low serum 25(OH)D concentrations are associated with increased risk for common respiratory infections [27]. Thus, differences in vitamin D concentrations between cervid species may influence pathogen susceptibility and immune biases.

Together, these findings indicate that vitamin D deficiency is associated with season (winter, short day length) and age in WTD. The caveat to this supposition, however, is that a clear definition of deficiency in WTD should be defined based upon impairment of relevant functional biomarkers of activity. These would include–but not be limited to–bone density measurements in adults, and rickets in the young while correlating extremely low 25-(OH)D measurements with the development of subclinical hypocalcemia. To define immune system biomarkers of sub-clinical (i.e. not metabolic) vitamin D deficiency in WTD, *in vitro* studies are planned to evaluate immunological ramifications associated with natural variations in vitamin D status, as defined in the present study. Additional feed trials will be required to provide recommendations for vitamin D supplementation within cervid diets. Present findings provide an approximate range of 25(OH)D concentrations within WTD for future trials.

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W. Ray Waters, DVM, PhD

National Animal Disease Center
2300 Dayton Avenue
Ames, Iowa 50010
USA
Telephone: +1 (515)-663-7756
Fax: +1 (515)-663-7458
E-mail: ray.waters@ars.usda.gov