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Serum Reference Intervals of Fat-soluble Vitamins D and E in Wild Florida Manatees (*Trichechus manatus latirostris*) and Possible Implications for the Health of Manatees in Managed Care

Ray L. Ball[1,2,3] [1]ZooTampa at Lowry Park, 1101 W Sligh Avenue, Tampa, Florida 33604, USA; [2]Current address: Eckerd College, Natural Sciences Collegium (NAS), James Center for Molecular and Life Sciences – 096, 4200 54th Avenue S, St. Petersburg, Florida 33711-4700 USA; [3]Corresponding author (email: ballrl@eckerd.edu)

ABSTRACT: Serum levels of alpha-tocopherol (vitamin E) and 25-hydroxyvitamin D (vitamin D) were measured in 25 wild Florida manatees (*Trichechus manatus latirostris)* from Crystal River National Wildlife Refuge, Citrus County, Florida, US. The samples were collected during the 2017 and 2018 winter health assessments. Reference intervals for alpha-tocopherol were 0.15–3.2 μg/L with mean value of 0.76 μg/mL and for 25-hydroxyvitamin D were 4.3–23 nmol/L with a mean of 13.88 nmol/L. Four, long-term captive manatees and nine manatees in rehabilitation were available for comparisons. Alpha-tocopherol levels were within the established range for free-ranging manatees. Mean 25-hydroxyvitamin D levels in long-term captive and manatees in rehabilitation fell well outside the reference interval for free-ranging manatees, being six- to sevenfold higher. The absence of significant differences in alpha-tocopherol between wild and captive manatees may not have any significant direct clinical implications but may warrant an investigation into other antioxidant mechanisms in manatees. The elevated 25-hydroxyvitamin D levels seen in captivity were unexpected and, in a few individuals, may pose a health challenge. Calcium is known to be elevated in captive manatees as well; a close examination of a potential hypervitaminosis D and hypercalcemia may be warranted.

Key words: 25-hydroxyvitamin D, alpha-tocopherol, Florida manatees, *Trichechus manatus latirostris,* vitamin D, vitamin E.

Free-ranging manatees consume various forms of submerged aquatic vegetation, including grasses and algae that are higher in plant fiber and ash content than is romaine lettuce (*Lactuca sativa* L. var. *longifolia*; Siegal-Willott et al. 2010). Captive manatees are fed primarily romaine lettuce, which is sometimes mixed with raw vegetables, fruit, and processed primate chows, but the nutrient content has not been completely characterized (Harshaw 2012). Concerns have been raised that these diets may predispose captive manatees to obesity, diabetes mellitus, dysbiosis, thermoregulatory disorders, and other conditions, as has been seen in other herbivores (Siegal-Willott et al. 2010). Obesity and diabetes mellitus have been documented in both manatees in rehabilitation (RH) and long-term captive (LTC) manatees (Ball 2019). Nutrient analysis of feedstuffs is one essential component of providing appropriate nutrition to any animal, and selected feedstuffs have been analyzed for captive manatees (Siegal-Willott et al. 2010). Nutritional assessment of manatees themselves has been limited to nutrient intake studies evaluating crude fiber, crude protein, ash, nonacid digestible fiber, acid digestible fiber, and dry matter in the diet (Harshaw 2012), as well as numerous trace minerals in both wild and captive manatees (Takeuchi et al. 2016). Circulating fat-soluble vitamins have not been previously examined in either wild or captive manatees. This report describes the serum levels of the fat-soluble vitamins alpha-tocoperol and 25-hydroxyvitamin D from wild Florida manatees as well as RH manatees and several LTC manatees.

Serum samples were collected from 25 wild manatees from the Crystal River (CR) National Wildlife Refuge, Citrus County, Florida, US (28°53′01.6″N, 82°35′33.1″W) as part of the health assessments during December 2016 through February 2017 and December 2017 through February 2018. Routine health assessments were performed by, and under the authority of, the US Geological Survey Sirenia Project and Florida Fish and Wildlife Conservation Commission using methodology previously reported (Bonde et al. 2012). Blood was collected from the brachial plexus, serum was separated at the capture site, and aliquots

TABLE 1. Descriptive statistics and 90% confidence intervals (CI) of alpha-tocopherol and 25-hydroxyvitamin D from wild Florida manatees (*Trichechus manatus latirostris*). Samples were collected as part of routine health assessment of manatees in Crystal River, Citrus County, Florida, USA, December 2016 through February 2017 and December 2017 through February 2018. N = number of manatees sampled.

	Alpha-tocopherol (µg/L)	25-hydroxyvitamin D (nmol/L)
N	24	24
Mean	0.76	13.88
Median	0.56	13.50
SD	0.70	4.41
Minimum	0.10	6.00
Maximum	3.58	21.00
90% CI for lower limit	0.15	4.3
90% CI for upper limit	3.2	23

were refrigerated until the samples were placed in a –80 C freezer later on the day of collection. All manatees were released uneventfully after standard biologic and biomedical data were collected. These samples were submitted to the Michigan State University Veterinary Diagnostic Laboratory for 25-hydroxyvitamin D and alpha-tocopherol determination. Mean, median, standard deviation, and maximum and minimum values were calculated using untransformed data. Ninety percent confidence intervals, as defined by the American Society for Veterinary Clinical Pathology (Friedrichs et al. 2012), were calculated using Box Cox transformed data. Descriptive statistics were compiled using Reference Value Advisor freeware (Geffré et al. 2011). For both 25-hydroxyvitamin D and alpha-tocopherol, there was one individual manatee that was an outlier for each vitamin; reference ranges were calculated without this outlier (n=24). All data are summarized in Table 1. Reference intervals for alpha-tocopherol were 0.15–3.2 µg/mL with mean value of 0.76 µg/mL and for 25-hydroxyvitamin D were 4.3–23 nmol/L with a mean of 13.88 nmol/L. Available serum samples from LTC manatees, held in captivity for greater than 5 yr at a single institution, and RH sampled just prior to release from a critical care facility, had alpha-tocopherol values within the reference intervals of the wild CR manatees (LTC mean 1.51 µg/mL, n=4; RH mean 1.15 µg/mL, n=9). Average duration of hospitalization for all admission causes for RH at this care facility during this period was 151 d.

Alpha-tocopherol is an important antioxidant in many species but its significance in manatees is not known. Vitamin E is predominately found in the roots of seagrasses and may not always be consumed with the typical foraging behavior of wild Florida manatees, as compared to dugongs that actively excavate roots of vascular plants while foraging (Marsh et al. 2011). Serum selenium concentration has been noted to be lower in RH and LTC manatees compared to wild manatees (Takeuchi et al. 2016). The LTC manatees sampled in this study and by Takeuchi are from the same facility and house obese manatees. This obesity and subsequent chronic inflammation may be part of the reason that serum selenium is low, in spite the fact that serum alpha-tocopherol concentration is statistically not different from that of wild manatees. Selenium supplementation may be a useful adjunct to rehabilitated manatee care instead of alpha-tocopherol, but other antioxidants, such as vitamin C, also may have clinical potential.

It is known that 25-hydroxyvitamin D has numerous roles, including calcium and phosphorus metabolism as well as immune function in many species, but its significance in manatees is not known. Prolonged indoor housing could be problematic during rehabilitation of manatee calves, as has been demonstrated in young rehabilitating sea turtles with hypovitaminosis D (Bloodgood et al. 2018). The captive manatees in this study were all housed outdoors and estimated to be at least 2 yr of age. Given the role of sunlight in vitamin D metabolism, the nature of housing (indoors vs. outdoors) may be relevant to the health of manatees in human care. Younger manatees, or any manatee housed indoors, may warrant examination regarding the status of 25-hydroxyvitamin D.

Several individual LTC and RH manatees in this study had 25-hydroxyvitamin D values 10 times greater than the mean for wild CR manatees. Vitamin D2 (ergocalciferol) and 25-hydroxyvitamin D content of romaine lettuce is negligible (US Department of Agriculture 2019); the source for the elevated 25-hydroxyvitamin D measured in this study is currently unknown and warrants continued investigation. Captive manatees have higher serum levels of calcium than do wild manatees (Harvey et al. 2007). This suggests conversion of 25-hydroxyvitamin D to 1, 25 di-hydroxyvitamin D in the kidneys, as is expected in mammals, but an additional source of added 25-hydroxyvitamin D appears likely given the elevated levels seen in the RH and LTC manatees compared to the wild individuals. Excretion of 25-hydroxyvitamin D by macrophages, which contain the α1-hydroxylase enzyme, has been postulated to be the mechanism behind hypercalcemia in dogs with granulomatous disease (Mellanby et al. 2006). Similar elevations in 25-dihydroxyvitamin D are noted in the progression of tuberculosis in a population of people in an equatorial country (Owolabi et al. 2016). Without evidence of an increased consumed source of vitamin D, an endogenous source must be considered in these captive manatees. Chronic inflammation from wounds, tissue necrosis as is the case in cold stress syndrome, or brevetoxin exposure are possible, but all captive manatees sampled in this study were no longer exposed to any of these hazards. Chronic abscess formation or developing tissue scarring and mineralization are certainly possible and could be a source of granulomas. Other anthropogenic sources of such potential inflammation may need serious consideration, and these include nutrition and feeding practices of manatees in managed care.

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LITERATURE CITED

Ball RL. 2019. Nutritional concerns of rehabilitated and long term captive wild Florida manatees (*Trichechus manatus latirostris*) with reference to healthy wild populations. In: *Proceedings of the European Association of Zoo and Wildlife Veterinarians conference*, Kolmarden, Sweden, 12–15 June; Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany, p. 74.

Bloodgood JCG, Norton TM, Hoopes LA, Hernandez SM. 2018. Blood nutritional markers reveal effects of rehabilitation diet on green sea turtle health. In: *Proceedings of the 67th annual International Wildlife Disease Association conference*, St. Augustine, Florida, 4–9 August; Wildlife Disease Association, Lawrence, Kansas, p. 40.

Bonde RK, Garrett A, Berlanger M, Askin N, Tan L, Wittnich C. 2012. Biomedical health assessments of the Florida manatee in Crystal River—Providing opportunities for training during the capture, handling, and processing of this unique aquatic mammal. *J Mar Anim Ecol* 5(2):17–28.

Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, Blanco-Chavez J. 2012. ASVCP reference interval guidelines: Determination of de ovo reference intervals in veterinary species and other related topics. *Vet Clin Pathol* 41:441–453.

Geffré A, Concordet D, Braun J-P, Trumel T. Reference Value Advisor: A new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. *Vet Clin Pathol* 40:107–112.

Harshaw LT. 2012. *Evaluation of the nutrition of Florida manatees (*Trichechus manatus latirostris*)*. PhD Thesis, University of Florida, Gainesville, Florida, 192 pp.

Harvey JW, Harr K, Murphy D. 2007. Clinical biochemistry in healthy manatees (*Trichechus manatus latirostris*). *J Zoo Wildl Med* 38:269–279.

Marsh H, O'Shea TJ, Reynolds JE III. 2011. *Ecology and conservation of the Sirenia: Dugongs and manatees*. Cambridge University Press, Cambridge, UK, 536 pp.

Mellanby RJ, Mellor P, Villiers EJ. 2006. Hypercalcaemia associated with granulomatous lymphadenitis and elevated 1,25 dihydroxyvitamin D concentration in a dog. *J Small Anim Pract* 47:207–212.

Owolabi O, Agbla S, Owiafe P, Donkor S, Togun T, Sillah AK, Ota MO, Sutherland JS. 2016. Elevated serum 25-hydroxy (OH) vitamin D levels are associated with risk of TB progression in Gambian adults. *Tuberculosis (Edinb)* 98:86–91.

Siegal-Willott JL, Harr K, Hayek L-AC, Scott KC, Gerlach T, Sirois P, Reuter M, Crewz DW, Hill RC. 2010. Proximate nutrient analyses of four species of submerged aquatic vegetation consumed by Florida manatee (*Trichechus manatus latirostris*) compared to romaine lettuce (*Lactuca sativa* var. *longifolia*). *J Zoo Wildl Med* 41:594–602.

Takeuchi NY, Walsh MT, Bonde RK, Powell JA, Bass DA, Gaspard JC, Barber DS. 2016. Baseline reference range for trace metal concentrations in whole blood of wild and managed West Indian manatees (*Trichechus manatus*) in Florida and Belize. *Aquat Mamm* 42:440–453.

US Department of Agriculture. 2019. *FoodData central*. https://fdc.nal.usda.gov/. Accessed May 2020.

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