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Regional Copper Deficiency in White-Tailed Deer

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Abstract

Minerals are critical in maintaining health and physiological function in wildlife. Geographic variation in soil and forage mineral concentration may predispose wildlife to mineral imbalances, where a common symptom is restricted somatic growth. We investigated if mineral imbalances could explain localized differences in morphology of white-tailed deer (*Odocoileus virginianus*) occurring in geographically proximate sites with similar management, climate, and habitat. We collected serum samples and morphological measurements from free-ranging white-tailed deer captured during 2011-2019 from coastal and inland rangeland sites in South Texas, USA. We measured mineral concentrations in serum from captured deer at each location. Asymptotic deer body mass and antler size averaged 8% - 20% smaller for deer at the coastal compared to the inland site. The proportion of deer with deficient levels of serum copper was greater at the coastal site (66% versus 14%). Our results suggest regional mineral deficiencies in deer may limit antler and body development. Wildlife managers should be aware of all aspects of wildlife nutrition and the importance of considering nutrients beyond energy and protein.

Keywords

Copper, Minerals, Nutrition, Ungulates, Serum, White-Tailed Deer

1. Introduction

Inorganic elements and compounds that play a role in animal growth and metabolism are termed minerals. Minerals are critical to physiological processes of animals, including mineralization of bones, enzyme and protein production, osmotic control, immune system response, and cellular respiration [1] [2]. In a biological context, minerals are usually categorized as macro- and micro-, or trace, minerals. Macrominerals are required in dietary concentrations >100 ppm, whereas trace minerals are needed in lesser quantities.

Despite the importance of minerals in the diet, mineral requirements for most species of wildlife are poorly documented [3]. Over 60 different elements have been documented in the mammalian body [4], but it is unclear what, if any, purpose many of these minerals serve. Furthermore, there are discrepancies in the literature on how many minerals are essential in the diet [1] [5] [6]. Overall, our understanding of mineral requirements in wildlife is hindered by complex interactions among mineral, environmental, and physiological factors [7].

When animals are unable to meet nutrient requirements, their physiological function may be negatively impacted. Extreme deficiencies may lead to mortality, but most cases of deficiency are less severe and are often manifested in rough coat or hair, anemia, lowered immunity, and general unthriftiness [1] [8]. Geographic variation in mineral availability can predispose certain populations to subclinical mineral deficiencies [2]. Some regional mineral deficiencies of ungulates are well documented and easily diagnosed from clinical symptoms [9]. However, other regional mineral deficiencies can go largely unnoticed, given their lack of definitive symptoms [2] [9] [10].

There is a growing body of work showing intraspecific ungulate morphology is correlated with soil and vegetation at a regional scale [11]-[16]. These regional size differences in ungulate morphology are thought to be nutritionally mediated, but a direct link with forage composition is largely unknown. Much research on regional differences in ungulate body and ornament size (*i.e.*, horns and antlers) focuses on macronutrients, such as energy and protein [17] [18] [19], even though a common symptom of mineral deficiencies is stunted growth [20]. This is not altogether surprising, given the scarcity of information on diagnosing mineral deficiencies in wildlife [9] [21]. White-tailed deer (*Odocoileus virginianus*) are well studied but only limited baseline values for diagnosing mineral abnormalities have been published [1]. Any effects of mineral deficiencies on size variation are unknown.

The goal of our study was to further our knowledge on the impacts of mineral health on the morphology of free-ranging populations of white-tailed deer. Specifically, we evaluated the hypothesis that morphological size differences of deer are associated with mineral deficiencies. In South Texas, deer found in the Coastal Sand Plain ecoregion appear physically smaller than deer in neighboring regions with less sandy soils. We predicted that deer from the site with smaller average deer morphology sizes would have higher incidence of serum mineral deficiencies.

2. Materials and Methods

2.1. Study Area

Our study occurred on two rangeland sites in South Texas (**Figure 1**). The El

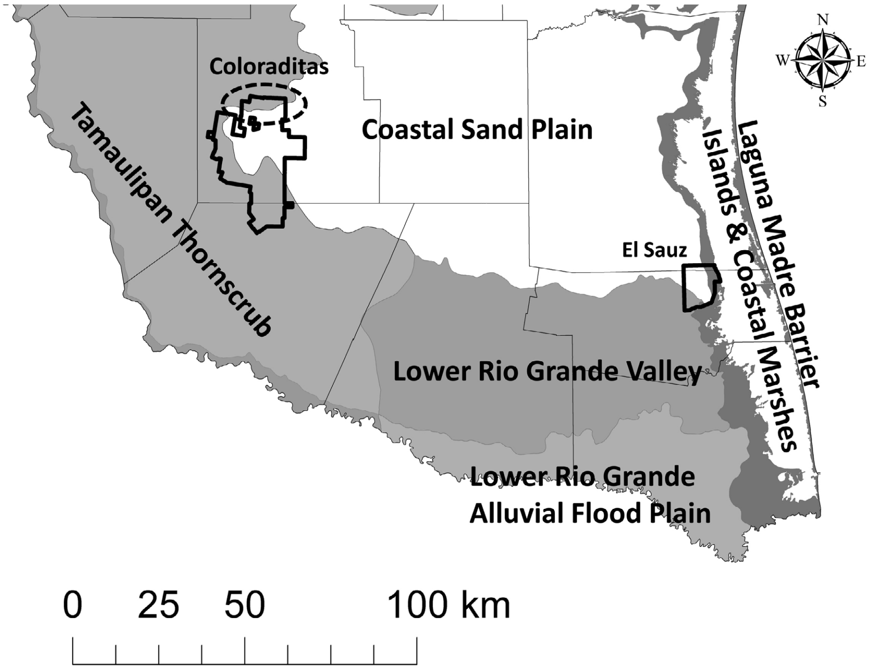


Figure 1. The coastal El Sauz Ranch and the inland Coloraditas grazing research and demonstration area on the San Antonio Viejo Ranch, located in South Texas, USA.

Sauz (hereafter, coastal) site was in Willacy and Kenedy Counties, Texas, USA (26˚34'42.7"N, 97˚32'14.52"W) and bordered the Gulf of Mexico. Mean annual precipitation in Willacy County was 66 cm; average annual high and low temperatures were 29.4˚C and 16.7˚C, respectively (1981-2010) [22]. The coastal site was located at the confluence of 3 level IV ecoregions [23]. Approximately 60% of the 10,984 ha was on the Coastal Sand Plain, while the remainder was in the Laguna Barrier Islands and Coastal Marshes and Lower Rio Grande Valley [23]. The second site, the Coloraditas Grazing Research and Demonstration Area (hereafter, inland site) was located on the San Antonio Viejo Ranch in Jim Hogg County, Texas, USA (27˚01'55.6"N, 98˚45'51.9"W). Mean annual precipitation in Jim Hogg County was 61 cm, and average annual high and low temperatures were 28.9˚C and 16.0˚C respectively (1981-2010) [22]. The inland site was comprised of 7,502 ha of native rangeland located on the transition zone between the Coastal Sand Plain and Texas-Tamaulipan Thornscrub level IV ecoregions [23]. The sites were operated as working cattle ranches and white-tailed deer inhabiting these properties were not hunted or managed.

2.2. Data Collection

We captured deer during October and November annually from 2011 to 2019 using the helicopter net-gun method [24] [25]. Deer were captured randomly as encountered without regard for age or sex. We estimated age of deer, in 1-year increments up to ≥6.5 years of age, based on tooth replacement and wear [26]. For deer that had been previously captured as a fawn or yearling (0.5 or 1.5 yr.) we corrected ages based on these previous known ages, as assigning age using tooth eruption patterns is more accurate than ages derived from tooth wear (≥2.5 yr. for white-tailed deer) [27]. We collected age-specific morphology measurements to quantify differences in antler size, body mass, and skeletal size. We quantified antler size using the gross Boone and Crockett score [28]. Hind foot length was measured from the top of the keratinized hoof to the posterior end of the tuber calcis and was used as an index of skeletal size [29]. All deer were weighed using a platform scale to the nearest 0.45 kg. Twenty mL of blood was drawn from the jugular vein of each deer using an 18-gauge × 2.54 cm blood draw needle (20-gauge × 2.54 cm for 0.5-year-old deer) and 10-mL, untreated vacuum tubes (Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ). Collected blood was either spun in the field or kept on ice until the samples could be processed, and all samples were spun ≤12 hr following collection. We centrifuged blood at 1,808 × g (3,500 rpm) until serum and red blood cells separated. Serum was aspirated via pipette into cryogenic vials and stored at −20˚C until analysis. All capture methods followed the American Society of Mammalogists guidelines [30] and were approved by Texas A&M University-Kingsville Institutional Animal Care and Use Committee (2017-09-22).

We selected a subset of 45 serum samples to have analyzed for mineral content from a single year (2015) to account for the potential confounding effect of year and due to funding constraints. We used multiple independent laboratories (Texas Research Institute for Environmental Studies, Huntsville, Texas, USA, and Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, Texas, USA) to measure mineral concentrations in serum with inductively coupled plasma mass spectrometry. Minerals measured included calcium, chloride, cobalt, copper, iron, potassium, manganese, magnesium, molybdenum, phosphorus, selenium, sodium, sulfur, and zinc and varied with testing capabilities of the laboratories contracted.

2.3. Statistical Analyses

To test for differences in deer morphology between sites, we fitted separate age-specific von Bertalanffy growth curves for body mass, hind foot length, and antler size (males only) for each site and sex [31]. We compared differences in asymptotic sizes between sites using a Welch's t-test. We used Fisher's exact tests to determine if the proportion of deer with serum mineral abnormalities differed between sites. A Fisher's exact test gives the exact hypergeometric probability, therefore, neither a test statistic nor degrees of freedom are reported for this statistical test. All statistical tests were performed in the R computing environment [32], with the aid of the FSA [33] package.

3. Results

Asymptotic body mass and hind foot length, for both females and males, differed between sites (**Table 1**). Asymptotic body mass of female deer at the inland site was 10% larger than the coastal site ($t_{329.08} = -149.42$, $P < 0.001$; **Figure 2**;

Table 1. Estimates produced by von Bertalanffy growth curves for body mass (kg), hind foot length (cm), and antler size (cm; calculated as gross Boone and Crockett score) for female and male white-tailed deer captured at the coastal and inland sites in South Texas, USA, from 2011 to 2019; L_∞ is the asymptotic morphological size estimate.

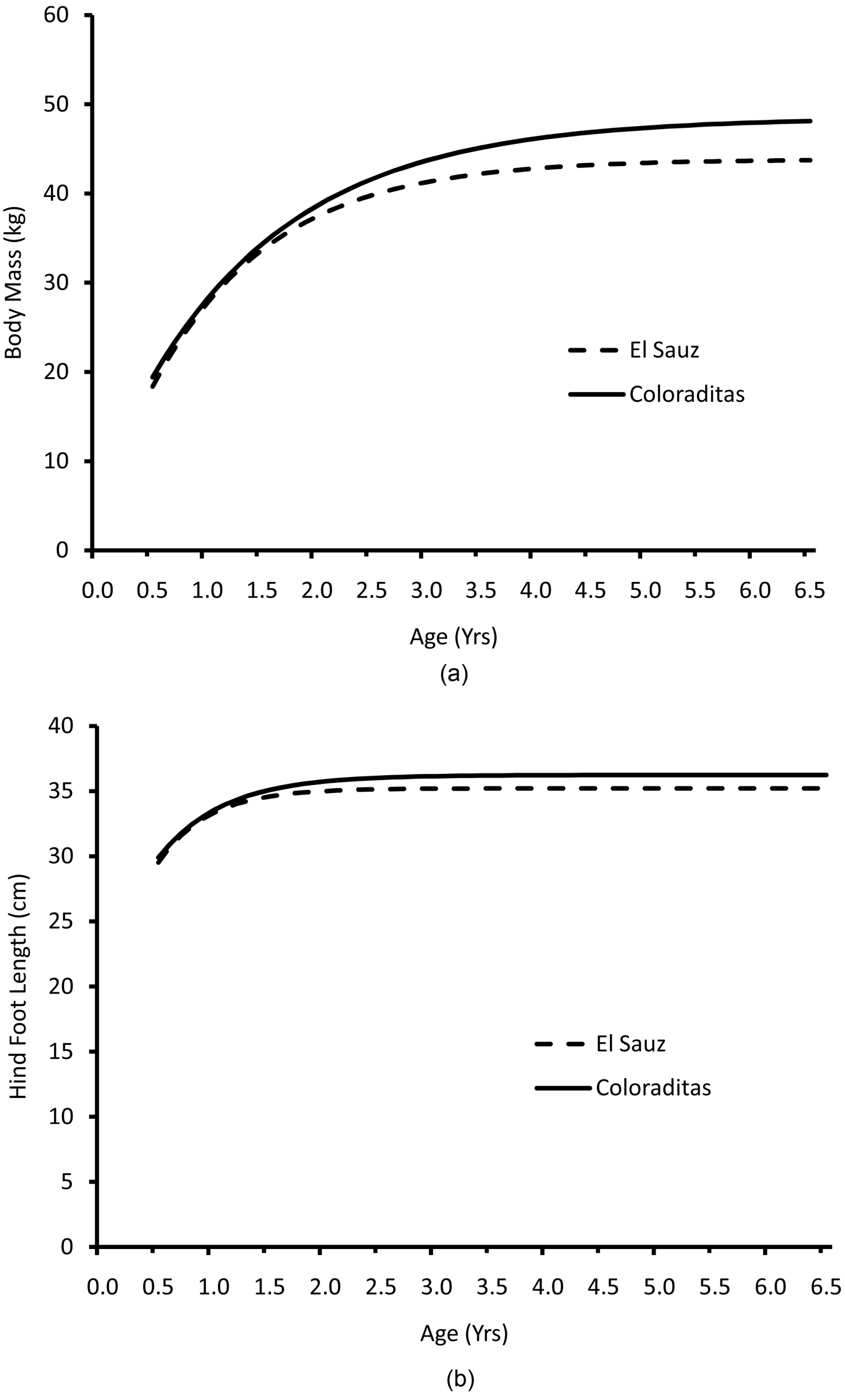
Site	Sex	Measurement	*n*	L_∞ ± SE (95% CI)
Coastal	Female			
		Body Mass	744	43.83 ± 0.009 (43.35 - 44.31)
		Hind Foot Length	746	35.21 ± 0.002 (35.10 - 35.32)
	Male			
		Body Mass	462	69.32 ± 0.052 (67.28 - 71.88)
		Hind Foot Length	469	37.38 ± 0.004 (37.21 - 37.56)
		Antler Size	406	334.62 ± 0.654 (310.99 - 373.16)
Inland	Female			
		Body Mass	318	48.51 ± 0.030 (47.52 - 49.57)
		Hind Foot Length	318	36.23 ± 0.005 (36.07 - 36.40)
	Male			
		Body Mass	389	86.78 ± 1.225 (82.78 - 91.66)
		Hind Foot Length	390	38.88 ± 0.004 (38.71 - 39.03)
		Antler Size	359	360.82 ± 0.634 (342.77 - 390.52)

Table 1). Hind foot asymptotic length was 3% and 4% greater at the inland as compared to the coastal site for females ($t_{355.67} = -189.41$, $P < 0.001$) and males ($t_{857.00} = -265.17$, $P < 0.001$), respectively. Male deer achieved an asymptotic body mass ($t_{388.00} = -14.24$, $P < 0.001$) and antler size ($t_{760.12} = -28.76$, $P < 0.001$) 20% and 8% greater at the inland than the coastal site, respectively (Figure 3).

Sixteen of 24 (67%) deer captured at the coastal site had deficient copper serum levels, and only 3 of 21 (14%) of the deer caught at the inland site were deficient (0.67 versus 0.14, $P < 0.001$, Fisher's exact test, Table 2). Seven of 21 (33%) deer at the inland site had higher than normal serum potassium levels, whilst none of the deer at the coastal site (0%) deviated from the normal range (0.33 versus 0.00, $P = 0.003$, Fisher's exact test). No other differences in serum mineral abnormalities between sites were observed ($P \geq 0.07$, Fisher's exact test).

4. Discussion

All deer exhibited multiple serological mineral abnormalities, suggesting our current knowledge of normal ranges is incomplete. Mineral toxicities result in readily visible symptoms and often morbidity, while symptoms of deficiencies are less severe [20]. Therefore, we believe most of the discrepancies in serum mineral values we observed above the "normal range" reflect a lack of knowledge regarding mineral balances in wildlife, not true abnormalities. We encourage wildlife health practitioners to publish more normal mineral values as there is a

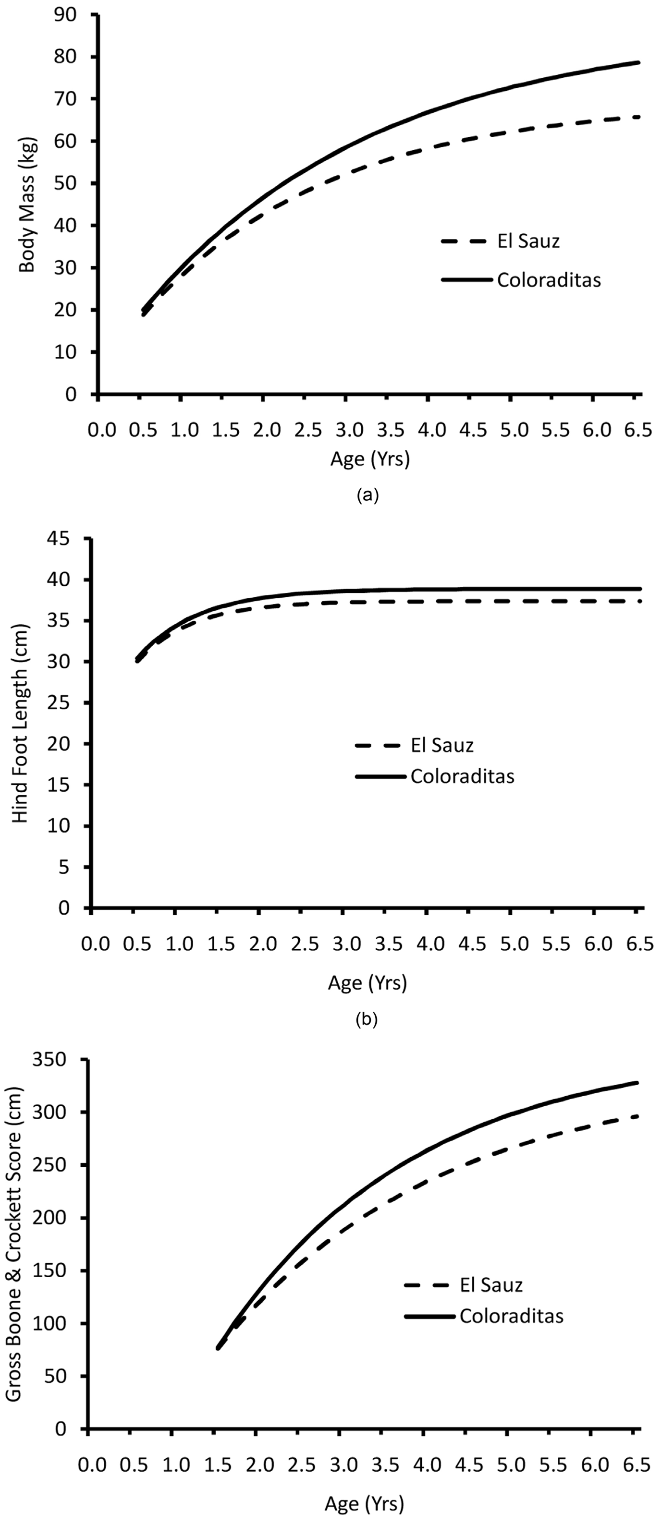


(a)

(b)

Figure 2. Von Bertalanffy growth curves constructed with morphology measurements collected from female white-tailed deer captured annually between 2011 and 2019. Mean asymptotic body mass (kg; (a)) and hind foot length (cm; (b)) at the inland Coloraditas site were 10% and 3% larger than those from the coastal El Sauz site, respectively.

consensus that current knowledge is lacking, yet little sharing of knowledge is evident. For the remainder of our discussion, we focus on the observed deficiencies, as these more likely are indicative of population mineral health and are often



(a)

(b)

(c)

Figure 3. Von Bertalanffy growth curves constructed with morphology measurements collected from male white-tailed deer captured annually between 2011 and 2019. Mean asymptotic body mass (kg; (a)), hind foot length (cm; (b)), and antler size (cm; (c)) at the inland Coloraditas site were 20%, 4%, and 8% larger than those from the coastal El Sauz site, respectively.

Table 2. Serum mineral concentrations for white-tailed deer captured at the coastal El Sauz site and inland Coloraditas site in South Texas, USA, during October and November 2015 and number of animals below or above the normal range (reference ranges are those used by the Texas A&M Veterinary Medical Diagnostic Laboratory for *Odocoileus* spp. and are based on Puls 1994, Mineral Levels in Animal Health and data collected by Tom Herdt (Nutritionist at the Michigan State University Veterinary Diagnostic Laboratory; precision of laboratory measures is represented by the decimal numbers supplied). Hyphens denote where data is unavailable due to a lack of reference values for these minerals.

Mineral (unit)	Normal Range	*P*-value	Coastal		Inland	
			Median (*n*)	Number below/above normal level	Median (*n*)	Number below/above normal level
Ca (mg/dL)	8.1 - 10.5	0.07	11.1 (24)	0/15	10.2 (21)	0/7
Cl (mEq/L)	93 - 103	0.41	99 (10)	0/0	99 (7)	0/1
Co (ng/mL)	-	-	0.31 (10)	-	0.37 (7)	-
Cu (ppm)	0.60 - 1.30	<0.001	0.20 (24)	16/0	1.55 (21)	3/0
Fe (ppm)	152 - 277	0.75	413 (24)	2/15	482 (21)	0/16
K (mEq/L)	3.6 - 9.6	0.003	5.11 (24)	0/0	6.96 (21)	0/7
Mg (mEq/L)	1.3 - 2.4	0.33	3.2 (24)	0/23	3.0 (21)	0/18
Mn (ng/mL)	-	-	3.57 (10)	-	2.81 (7)	-
Mo (ng/mL)	-	-	1.81 (10)	-	0.62 (7)	-
Na (mEq/L)	142 - 152	1.00	176 (24)	0/19	167 (21)	0/16
P (mEq/L)	5.9 - 10.3	0.74	15.6 (24)	0/18	14.7 (21)	0/14
S (ppm)	-	-	1065 (14)	-	1060 (14)	-
Se (ng/mL)	60 - 150	0.49	110.75 (10)	0/2	110.21 (7)	0/0
Zn (ppm)	0.50 - 1.00	1.00	0.4 (24)	21/0	0.4 (21)	19/0

manifested in the lowered growth that we observed.

Long-term trends on age-specific morphology show deer on the coastal site were physically smaller than deer on the inland site. Previous work suggests these observed size differences are nutritionally, rather than genetically, mediated [34]. Observational studies cannot prove causality, but the higher incidence of copper deficiency at the coastal site suggests copper might be limiting deer growth in coastal habitats. In wild ungulates, hypocuprosis most often results in suppressed growth, lowered reproductive output, and increased risk to secondary factors, such as predation and infectious diseases [2] [35]. Previous research has shown recruitment rates obtained from aerial surveys are lower at the coastal than at the inland site [36]. Furthermore, captive feeding trials corroborate that normal reference ranges for serum copper concentration in adult white-tailed deer are ~1 ppm [37]. Thus, we believe the copper deficiencies we report here are truly deficiencies and not a result of inaccurate reference values.

Copper deficiencies have been observed in many species of livestock and in humans [38]. Ruminants can experience either primary or secondary hypocuprosis [39]. Primary copper deficiencies are caused by inadequate dietary copper. It is unknown what level of dietary copper is optimal for white-tailed deer, however, for cattle, 10 ppm of dietary copper is sufficient [40]. Native range plants growing on our study areas, as well as throughout Texas generally have low levels of copper (≤6 ppm on average) which could predispose herbivores to copper deficiencies [41] [42]. Secondary deficiencies result from lowered copper absorption in the digestive tract. Interactions between minerals may hinder absorption of copper in ruminants [38] [39]. In the presence of elevated sulfur and molybdenum levels, thiomolybdates are formed [43] [44]. These thiomolybdates react with copper to form insoluble complexes that are indigestible [45]. Therefore, optimal diets for cattle should have a copper to molybdenum ratio around 6:1, as ratios ≤2:1 routinely cause hypocuprosis. Additionally, elevated levels of dietary sulfur can result in the formation of copper sulfide in the digestive tract, which reduces the absorption of copper [46]. There are no published normal ranges of molybdenum serum concentrations for white-tailed deer, so we were unable to determine if the proportion of deer with abnormal molybdenum serum levels differed between sites. The molybdenum concentrations in serum, however, were higher at the coastal site than the inland site.

Our data based on the animal as an indicator approach indicated that copper might be a limiting nutrient for white-tailed deer in coastal regions along the Gulf of Mexico. Observational studies, however, cannot show causality and further work in controlled settings are needed to fully describe the relationship between growth and copper deficiencies in white-tailed deer. White-tailed deer consume highly diverse diets [47] and copper concentrations vary by forage class [48]. It is possible that deer at the coastal site are consuming less dietary copper, or more molybdenum, as an artifact of maximizing intake of digestible energy, or some other nutrient. Given normal mineral concentrations are poorly documented in white-tailed deer, it is possible that the deer at our coastal site represent the lower end of acceptable copper serum levels. Even with these caveats, our study suggests trace minerals are relevant in wildlife management and discussions pertaining to regional nutritional differences should include aspects of mineral levels in the future.

5. Conclusion

All 45 deer we sampled exhibited serum mineral concentrations outside of the normal range. Therefore, it is likely many species of wildlife, including white-tailed deer, have broader normal ranges of serum mineral levels than previously documented. Incidence of copper deficiencies was greater at the site with the smallest average deer morphology and a common symptom of copper deficiencies is suppressed somatic growth. Bioavailability of copper depends on the chemical structure of the copper, interactions with numerous other minerals, and other environmental and physiological factors [49]. Although, copper is potentially limiting deer growth, we do not recommend simply providing copper supplementation, given the complexities involved in trace mineral absorption.

Rather we suggest managers should focus efforts on providing a diversity of palatable forage, allowing deer to self-regulate their dietary mineral intake.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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