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Determination of Blood Micromineral and Fat-Soluble Vitamin Values for White-tailed Deer

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ABSTRACT

This study was to determine baseline values for whole blood and serum micromineral and vitamin concentrations for white-tailed deer (*Odocoileis virginianus*) in an attempt to establish dietary requirements. Open does (n = 223) were sampled during fall breeding procedures. Captive-raised does housed at high fenced ranches (n = 3) throughout Texas were used. Blood samples were analyzed for micromineral levels (Co, Cu, Fe, Mn, Mo, Se, and Zn) and fat-soluble metabolites (vitamin A, vitamin E, and cholesterol). Age of the doe and ranch were used as main effects. Sampled averages were 6.31 ng/mL Co, 1.04 µg/mL Cu, 220.41 µg/mL Fe, 4.43 ng/mL Mn, 4.23 ng/mL Mo, 172.48 ng/mL Se, 0.54 µg/mL Zn, 275.25 ng/mL vitamin A, 1.80 µg/mL vitamin E, and 79.61 mg/dL cholesterol. Ranch played an important role in micronutrient levels, with the exception of cholesterol ($P = 0.26$). Micronutrient least squared means were affected by age for Se, Zn, and vitamin E ($P < 0.01$). Pregnancy status was determined (n = 93) via blood 30-37 d after breeding procedure. Females that became pregnant at initial breeding attempt had higher serum Zn ($P < 0.01$) and vitamin E ($P = 0.03$) levels. The establishment of circulating blood micronutrient levels will serve as a baseline for future white-tailed deer nutrient requirement research.

KEY WORDS: microminerals, trace minerals, fat-soluble vitamins, white-tailed deer

INTRODUCTION

White-tailed deer (*Odocoileus virginianus*) are a native Texas species that serve an important role in the ecosystem, economy, heritage, and more recently, agriculture sectors of Texas. Ranches dedicated to raising white-tailed deer have become a well-established portion of the agriculture sector. In the last decade, the scope of captive deer production has grown dramatically as producers continue to finetune their production methods to maximize profits. In the state of Texas alone, the white-tailed deer industry's overall economic impact exceeds $1.6 billion annually (Outlaw et al. 2017). Each year, the deer breeding industry is responsible for contributing $349.4 million to the Texas economy. There are approximately 1,006 permitted high-fenced deer ranches in the state that support countless jobs (Anderson et al. 2007). It is estimated that $786.9 million is spent on ranch operating expenses, 30% of which is feed and hay costs (Outlaw et al. 2017). Just like other livestock species, feed costs are a huge portion of an operation's monthly expenses. Producers understand that high quality feed ingredients are necessary to unlock the genetic potential of their herd, but in order to maximize profits feed resources must be allocated properly. White-tailed deer ranches are founded with the goal of producing genetically superior does and large bucks with striking antlers to either be sold as breeders or be used for trophy hunts. Due to nutrition's impact on antler growth, supplemental feed and mineral programs are implemented with zeal, as this is perceived to be an unofficial portion of the Boone and Crockett antler equation. The Boone and Crockett equation is the industry standard for measuring antler growth,

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rewarding racks for number of points, spread, width, length of tines, and circumference. However, there are many unknowns in regard to nutritional supplementation in deer as their base nutritional requirements are not fully understood. The deer industry still feeds animals using antiquated nutritional requirements based on other small ruminant species, such as sheep and goats, or field data which lacks scientific design. Very few large-scale nutrition focused research projects have been designed for white-tailed deer, in captivity or in the wild, leaving producers with little information when they build their feeding strategies. Furthermore, the research that has been completed is often discounted due to small sample sizes with a wide range of population variables.

Captive deer have drastically different behavioral patterns when compared to their wild relatives which may travel miles in search of specific browse plant species. Deer consume minute amounts of grass, preferring to select forbs and browse which are more nutrient dense (Wright et al. 2002). Due to the limited ranging capability of captive herds, offering a complete supplemental ration becomes essential to meet nutritional needs that may be lacking given the minimum browse variety typical of captive deer enclosures. Additionally, deer in captivity experience added immune system stress due to increased disease exposure through closer animal interaction (Bartoskewitz et al. 2007). Thus, research is needed to pinpoint the requirements of captive deer populations. However, it is inherently difficult to determine nutrient requirements for animals that are not contained in a dry lot setting and the methodology for feeding captive deer in this scenario has not been thoroughly developed as their livestock counterparts.

Forage and soil components, trace element interactions, genetic effect, physiological state, immune interaction, and rumen microflora are also influential variables that need to be accounted for in studies to come. There are few reports of blood analyses from free range or captive deer populations. Those that do exist are markedly small. Few studies have been completed that explore vitamin and micromineral profiles, specifically. The establishment of circulating blood micronutrient levels from this and subsequent studies will serve as a baseline for future white-tailed deer nutrient requirement research and feed formulation.

The objective of this study was to determine baseline values for whole blood and serum micromineral and vitamin concentrations for white-tailed deer in an attempt to establish dietary requirements of trace minerals, fat-soluble vitamins, and other metabolites.

MATERIALS AND METHODS

Ethical Statement. All care, handling, and sampling of deer was approved by the Sam Houston State University Institutional Animal Care and Use Committee (Protocol number: 19-12-04-1008-3-01) prior to the start of the experiment.

Data Collection. Captive raised, white-tailed does (n = 223) ranging in age from 1 to 9 yr were sampled. Samples were obtained from a combination of three independently managed ranches in the State of Texas. Deer were immobilized using a sedative, 1.5 cc Medetomidine Ketamine-HCl, administered via intravenous injection or restrained manually in a chute in conjunction with artificial insemination, embryo collection, and embryo transfer procedures. One 6 mL blood sample was collected from each doe via jugular venipuncture into a royal blue-top tube specifically for trace element analysis and containing an anticoagulant, ethylenediaminetetraacentic acid, for whole blood and serum analysis of microminerals. A second 6 mL blood sample was collected into lavender-top tubes, also containing ethylenediaminetetraacentic acid was used for harvesting of serum used in vitamin analysis (Vacutainer; Becton, Dickinson, and Co., Franklin Lakes, NJ). Following sample collection, 3 cc Atipamezole HCl was administered to each doe as sedative reversal. All animals were returned to the herd following sampling procedure. Samples were labeled using an animal identification number specific to this project to maintain anonymity. Birth dates were determined based on written records provided by each ranch. Age was calculated according to ranch recorded birth date and simplified to the nearest whole year.

Within 3 hrs of initial blood draw, blood was centrifuged for 20 minutes at 680 *g* upon which serum was extracted from the lavender top tubes and moved to plastic transport tubes. Whole blood and serum samples were chilled in coolers and shielded from light. Within 24 hr of collection, all samples were delivered to Texas Veterinary Medical Diagnostic Laboratory in College Station, TX for analysis of Co, Cu, Fe, Mg, Mo, Se, Zn, vitamin A, vitamin E, and cholesterol levels. Testing was performed according to Texas Veterinary Medical Diagnostic Laboratory standard operating procedures.

Pregnancy status (n = 93) was determined via blood test by ranch staff 30-37 d after breeding procedure. Pregnancy-associated glycoproteins (PAGs) found in blood served as indication of fetal growth. Positive pregnancy status refers to does who became pregnant following artificial insemination or embryo transfer procedures that occurred the day of sampling. Open pregnancy status refers to does that did not successfully conceive.

Data Analysis. All data were analyzed using the General Linear Model procedure of SAS (SAS Institute Inc., Cary, NC, USA) to determine average values and interaction between ranch, animal age, and pregnancy status. Each animal served as an experimental unit.

Study Area. All three ranches were located in the Inner Coastal Plains physiographic region. The Inner Coastal Plains physiographic region is marked by elevations between 90-245 m featuring a series of parallel ridges and valleys. Exact location of ranches was omitted to maintain anonymity, but Ranch A was located in the Brazos Valley, Ranch B in the Piney Woods, while Ranch C fell in the South Texas Plains subregion. Soil quality varied by ranch from sandy, traditionally nutrient-poor soils to areas of deep, acidic loams and areas of high clay and Fe content. The average long-term minimum and maximum temperatures during the white-tailed doe breeding season, spanning November – December, were 6 °C - 21 °C. The average daily precipitation in 2019 for all locations was lower than the long-term averages for the state of Texas.

All does were housed in a high-fenced setting, limited to white-tailed deer. This minimized interaction and competition with other species. Pen size and stocking density varied by ranch with Ranch A and B does confined to pens ranging from 0.20–0.81 hectares. Browse was minimal to nonexistent at both ranches. Ranch A pens had warm season grasses in doe pens while Ranch B was devoid of forages thus limiting the diet to provided feedstuffs only. Females were given access to alfalfa hay, textured feed, and a free choice, gravity fed pellet. Textured feed (Table 1) was handfed daily at varying quantities based on the condition of the animal. Ranches A and B used the same alfalfa hay supplier; thus, their hay was expected to have similar nutrient values (Table 1). Quantities consumed were not measured but could be an area of interest in future studies. Ranch C maintained the lightest stocking density of 1.10 hectares per doe. Ranch C does had access to a free choice, gravity fed pellet and textured feed fed daily from fawning through breeding. Ranch C differed in that does did not receive alfalfa hay, but females had access to naturally occurring, native browse species.

Table 1
Proximate analysis and mineral breakdown of daily hand fed, feedstuffs[a] at individual ranches plus alfalfa hay[b]

	Ranch A	Ranch B	Ranch C	Alfalfa Hay
Crude Protein, %	17.90	17.40	21.90	19.90
Ca, %	2.62	2.60	1.89	1.51
P, %	0.82	1.05	0.88	0.27
K, %	1.22	1.34	1.34	1.88
Mg, ppm	0.47	0.54	0.32	0.62
Na, ppm	2,361.00	2,977.00	2,080.00	163.00
Cu, ppm	50.00	81.00	58.00	13.00
Fe, ppm	64.00	69.00	50.00	13.00
Mn, ppm	320.00	368.00	67.00	52.00
Zn, ppm	284.00	318.00	258.00	14.00

[a]Quantities fed varied by ranch
[b]Alfalfa hay fed at Ranches A and B only

RESULTS

Study Sampled Averages. Table 2 contains previously accepted healthy micromineral blood serum ranges of various cervid species and classes established by Puls (1994), henceforth referred to as reference data. This data was built using a compilation of small, antiquated studies utilizing a variety of cervid species from various environments under a broad range of nutritional conditions. These ranges are currently used by veterinarians and diagnostic labs when evaluating cervid blood samples to determine health status and make nutritional recommendations. In this current study, sampled means fell within previously accepted ranges for all established metabolites with the exception of Se, which was 22.48 ng/mL higher in the trial data set than the reference data range of 60-150 ng/mL. Sampled averages (n=223) were 6.31 ng/mL of Co, 1.04 µg/mL of Cu, 220.41 µg/mL of Fe, 4.43 ng/mL of Mn, 4.23 ng/mL of Mo,

172.48 ng/mL of Se, 0.54 µg/mL of Zn, 275.25 ng/mL of vitamin A, 1.80 µg/mL of vitamin E, and 79.61 of cholesterol in the current study.

Table 2
Reference data averages (Puls, 1994) compared to current study LS Means of serum micromineral and fat-soluble metabolites in sampled does

Analyte	Reference Data Average Ranges	Current LS Mean	SE
Co (ng/mL)	Unknown	6.31	0.194
Cu (µg/mL)	0.60-1.30	1.04	0.012
Fe (µg/mL)	152.00-277.00	220.41	12.134
Mn (ng/mL)	Unknown	4.43	0.449
Mo (ng/mL)	Unknown	4.23	0.141
Se (ng/mL)	60.00-150.00	172.48	1.383
Zn (µg/mL)	0.50-1.00	0.54	0.010
Vitamin A (ng/mL)	Unknown	275.25	15.421
Vitamin E (µg/mL)	Unknown	1.80	0.055
Cholesterol (mg/dL)	Unknown	79.61	1.920

Ranch. Ranch A provided 99 hd, Ranch B had 98 hd, and 26 hd were sampled at Ranch C (Figure 1). There were significant differences ($P < 0.01$) between ranch for all measured microminerals and fat-soluble vitamins as noted in Table 3. Cholesterol levels, however, ($P = 0.26$) did not vary between ranches. Ranch C was statistically different from Ranches A and B for Cu, Fe, Mn, and vitamin A. However, Ranch A differed from Ranches B and C regarding Se and vitamin E. Zinc varied by ranch, with all locations being statistically different ($P < 0.01$).

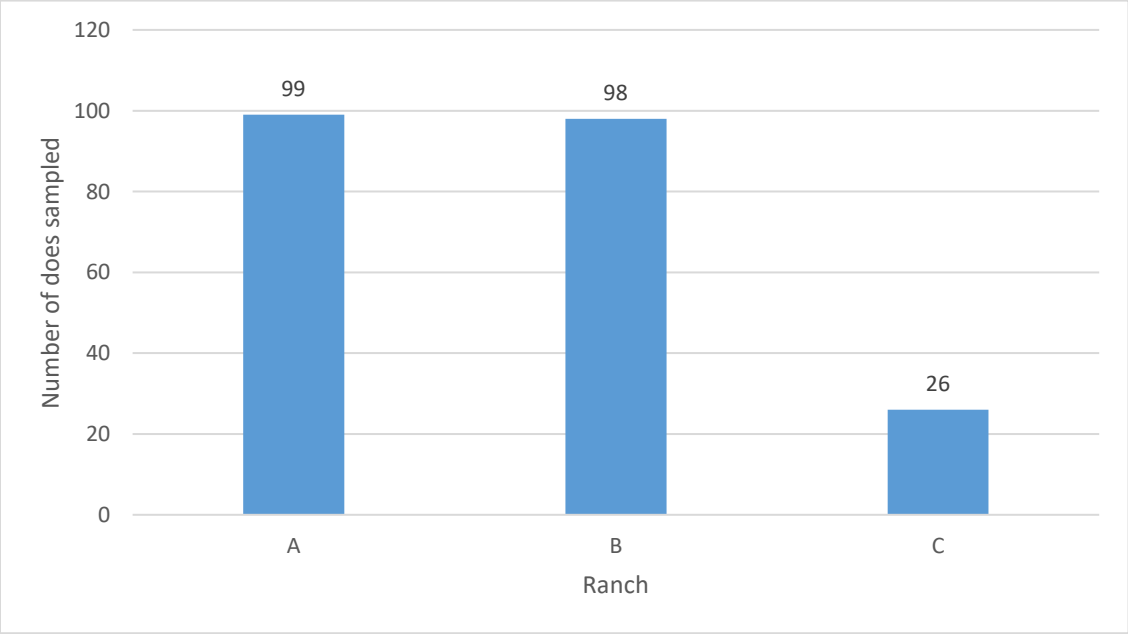

Figure 1. *Total number of does sampled by ranch (n = 223).*

Table 3. *LS Means of serum micromineral and fat-soluble metabolites in sampled does by ranch*

Analyte	Ranch A	Ranch B	Ranch C	SE[d]	P-Value
Cu (µg/mL)	0.99[a]	1.02[a]	1.33[b]	0.044	<.01
Fe (µg/mL)	179.72[a]	197.84[a]	431.90[b]	38.116	<.01
Mn (ng/mL)	2.45[a]	3.87[a]	13.79[b]	1.319	<.01
Se (ng/mL)	160.51[a]	180.34[b]	185.93[b]	3.810	<.01
Zn (µg/mL)	0.52[b]	0.47[a]	0.74[c]	0.035	<.01
Vitamin A (ng/mL)	283.61[b]	293.13[b]	40.40[a]	57.283	<.01
Vitamin E (µg/mL)	2.24[b]	1.53[a]	1.60[a]	0.155	<.01
Cholesterol (mg/dL)	75.21	79.28	86.63	6.218	0.26

[abc]Means with different superscripts differ at $P < 0.05$
[d]Pooled Standard Error of the Mean

Age. The number of does sampled within each age group were not proportional (Figure 2). White-tailed deer life expectancy is 6.5 yrs, thus does aged 6-9 yrs were combined to form an "aged" category (Lopez et al. 2003). Table 4 compared does in the study by age group. Age showed to play a significant role in Se, Zn, and vitamin E levels in white-tailed doe blood serum. As does aged, serum Zn levels decreased (Figure 3). No concrete trend was established for Se (Figure 4) and vitamin E (Figure 5) as does aged. Copper, Fe, Mn, vitamin A, and cholesterol did not show to be affected by age.

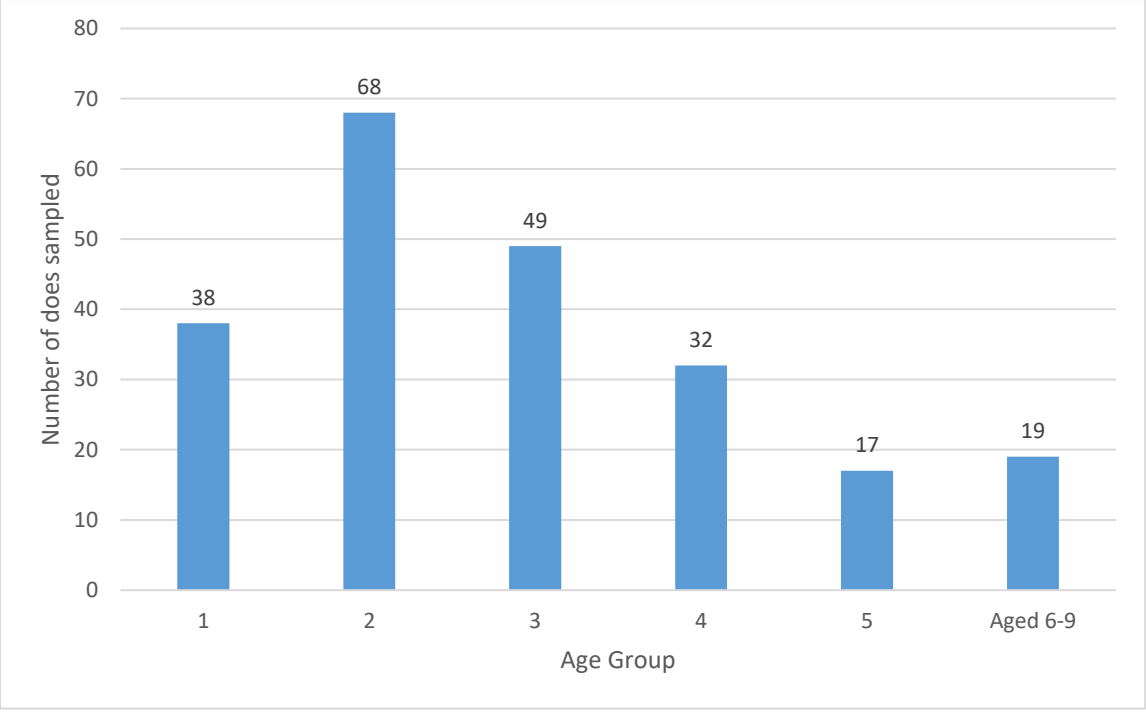

Figure 2. *Total number of does sampled by age (n = 223).*

Table 4. LS Means of serum micromineral and fat-soluble metabolites in sampled does by age group.

Analyte	1	2	3	4	5	6-9	SE[d]	*P*-Value
Cu (µg/mL)	1.07	1.13	1.15	1.08	1.12	1.13	0.053	0.32
Fe (µg/mL)	290.55	298.05	236.61	284.46	271.17	238.07	43.307	0.41
Mn (ng/mL)	7.22	6.38	7.23	7.23	6.20	5.96	1.495	0.93
Se (ng/mL)	163.39[a]	179.20[b]	177.80[b]	181.23[b]	176.36[b]	175.63[b]	4.331	<.01
Zn (µg/mL)	0.61[b]	0.63[b]	0.60[b]	0.58[ab]	0.51[a]	0.51[a]	0.030	<.01
Vitamin A (ng/mL)	165.82	224.48	270.83	201.72	175.24	196.18	56.030	0.40
Vitamin E (µg/mL)	2.12[c]	1.69[abc]	1.88[bc]	1.49[abc]	1.84[bc]	1.72[abc]	0.184	<.01
Cholesterol (mg/dL)	87.24	79.44	86.31	75.13	76.40	77.72	7.420	0.35

[abc]Means with different superscripts differ at $P < 0.05$
[d]Pooled Standard Error of the Mean

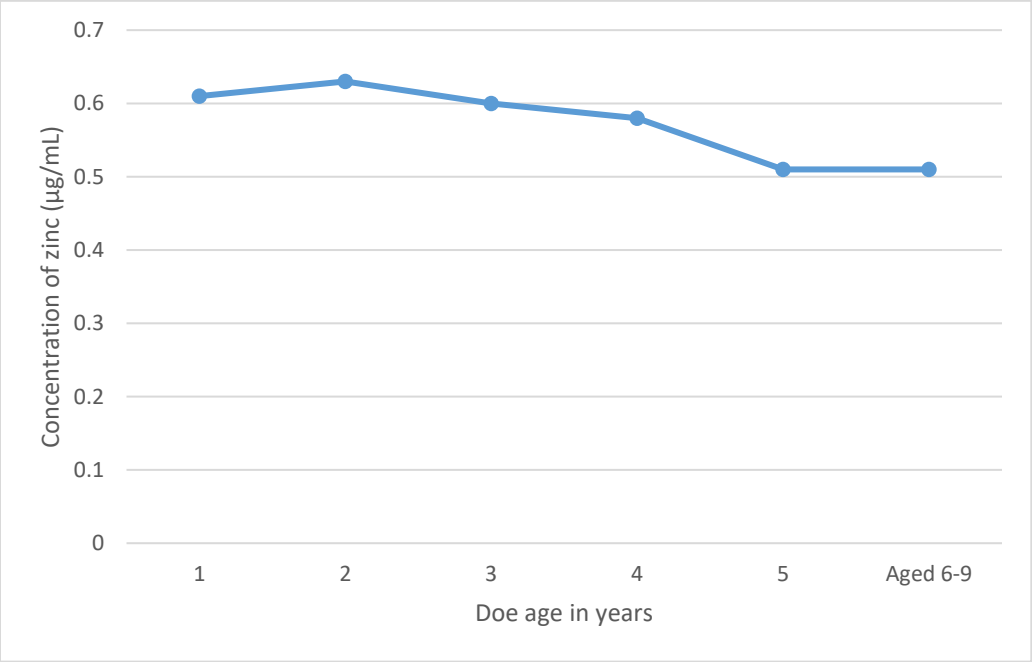

Figure 3. Zinc LS means by doe age group.

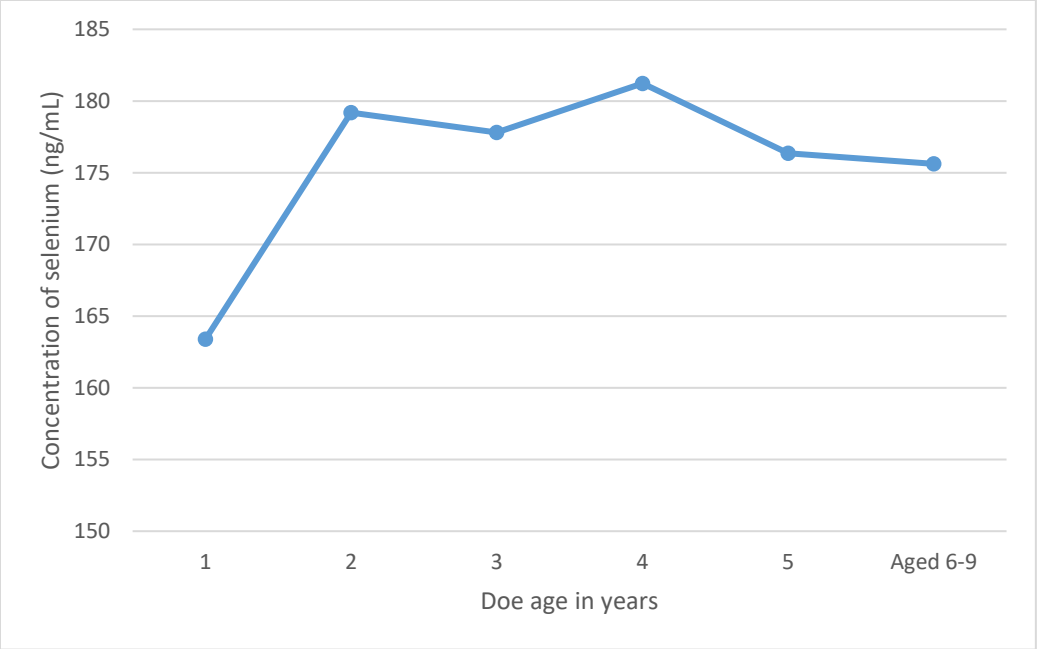

Figure 4. Selenium LS means by doe age group.

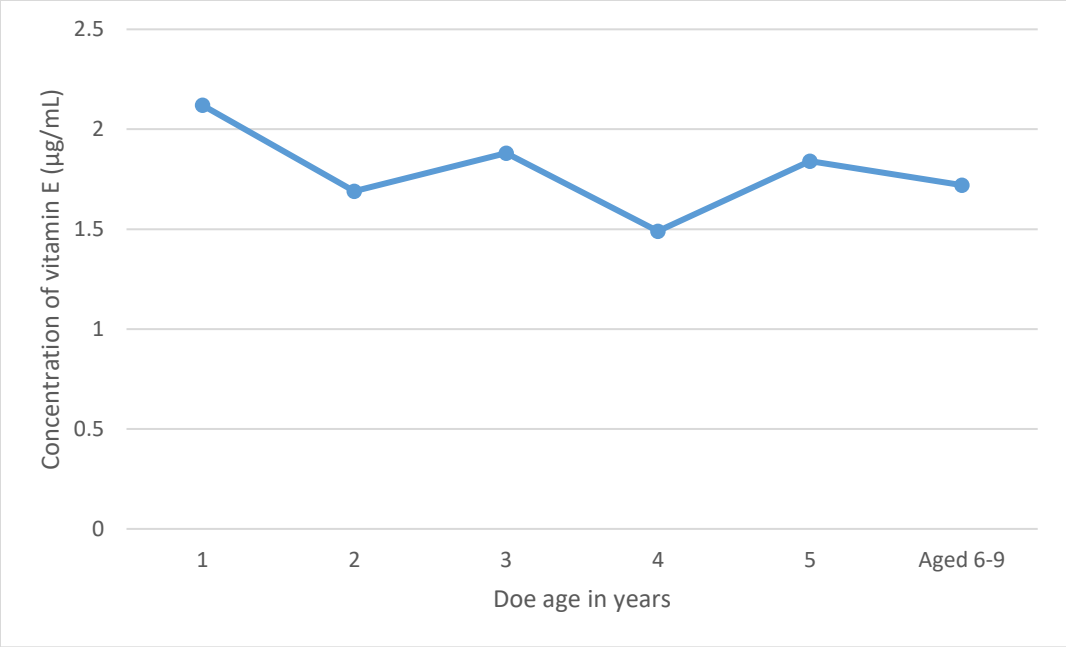

Figure 5. Vitamin E LS means by doe age group.

Interactions. Interactions between main effects of ranch and age were detected for Co and Mo. Ranch C was lower than Ranches A and B for Co, but no trend was established across all ages and ranches (Figure 6). Molybdenum tended to increase with age at Ranches A and B (Figure 7).

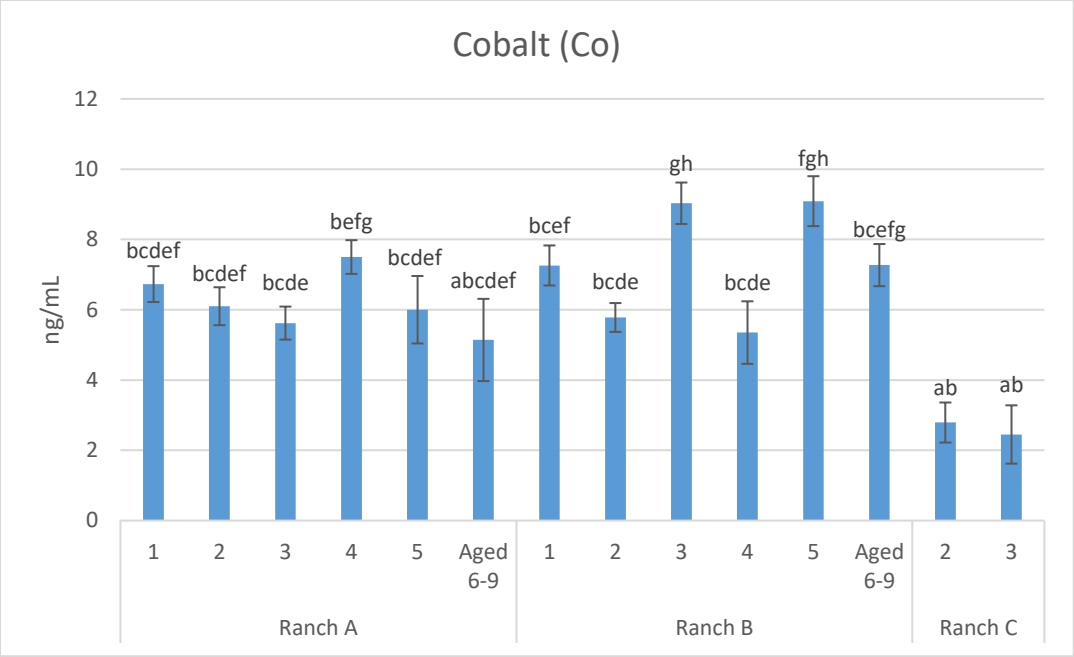

Figure 6. Interaction between main effects of age and ranch for Co.
[abcdefgh]Means with different superscripts differ at $P < 0.05$

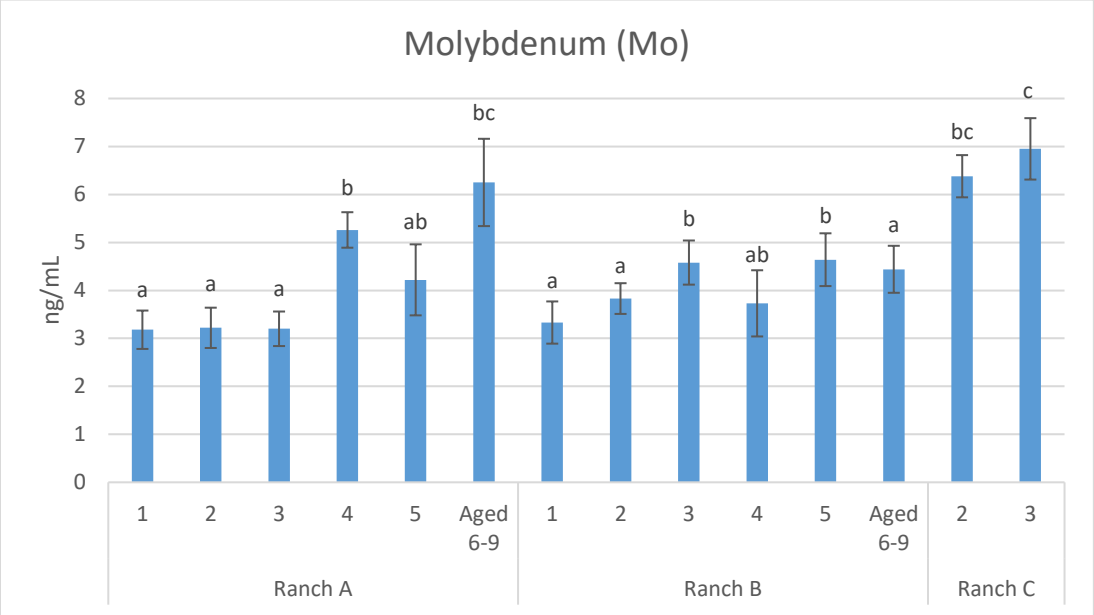

Figure 7. Interaction between main effects of age and ranch for Mo.
[abc]Means with different superscripts differ at $P < 0.05$

Correlation. No strong correlations were established across sampled analytes (Table 5). However, Fe and Zn had a weak correlation, 0.44. Similarly, Vitamin E and cholesterol also displayed a weak correlation, 0.44.

Table 5. Pearson Correlation Coefficients for serum micromineral and fat-soluble metabolites in sampled does.

	Co	Cu	Fe	Mn	Mo	Se	Zn	Vit A	Vit E	Cholesterol
Co (ng/mL)		-0.29	-0.16	-0.21	-0.06	-0.01	-0.17	0.15	0.27	0.21
Cu (µg/mL)	-0.29		0.14	0.36	0.25	0.31	0.19	-0.17	-0.10	0.18
Fe (µg/mL)	-0.16	0.14		0.08	0.21	0.19	0.44[a]	-0.03	-0.08	0.01
Mn (ng/mL)	-0.21	0.36	0.08		0.21	0.23	0.17	-0.16	-0.03	0.18
Mo (ng/mL)	-0.06	0.25	0.21	0.21		0.37	0.21	-0.10	-0.16	0.25
Se (ng/mL)	-0.01	0.31	0.19	0.23	0.37		0.17	-0.06	-0.39	0.21
Zn (µg/mL)	-0.17	0.19	0.44[a]	0.17	0.21	0.17		-0.04	-0.01	0.20
Vitamin A (ng/mL)	0.15	-0.17	-0.03	-0.16	-0.10	-0.06	-0.04		-0.03	-0.11
Vitamin E (µg/mL)	0.27	-0.10	-0.08	-0.03	-0.16	-0.39	-0.01	-0.03		0.44[a]
Cholesterol (mg/dL)	0.21	0.18	0.01	0.18	0.25	0.21	0.20	-0.11	0.44[a]	

[a]Denotes weak correlation

Pregnancy Status. Sampled LS means for does reported to be open following breeding procedures were statistically the same ($P > 0.05$) as bred females for all analytes with the exception of Zn and Vitamin E (Table 6). Open females had lower circulating Zn levels than bred does ($P < 0.01$). Vitamin E was also lower ($P = 0.03$) in females that did not successfully conceive on the first attempt.

Table 6. Pregnancy status of does determined by blood test 30-37 d following breeding procedure that occurred in conjunction with sampling for micromineral and fat-soluble analyte analysis.

Analyte	Open[a] LS Mean	Bred[b] LS Mean	SE[c]	*P* - Value
Co (ng/mL)	6.24	7.20	0.456	0.10
Cu (µg/mL)	0.99	1.00	0.031	0.95
Fe (µg/mL)	179.34	194.87	12.398	0.34
Mn (ng/mL)	4.31	4.02	0.384	0.56
Mo (ng/mL)	3.09	3.21	0.232	0.68
Se (ng/mL)	170.07	167.34	3.587	0.56
Zn (µg/mL)	0.42[x]	0.48[y]	0.025	<.01
Vitamin A (ng/mL)	270.56	271.38	15.387	0.97
Vitamin E (µg/mL)	1.78[x]	2.19[y]	0.151	0.03
Cholesterol (mg/dL)	77.09	80.38	4.803	0.61

[a]Open refers to does who did not became pregnant following artificial insemination or embryo transfer procedures that occurred the day of mineral sampling
[b]Bred refers to does who became pregnant following artificial insemination or embryo transfer procedures that occurred the day of mineral sampling
[c]Pooled Standard Error of the Mean
[xy]Means with different superscripts differ at $P < 0.05$

CONCLUSION AND DISCUSSION

Study Sampled Averages. Study averages validate the assumption that white-tailed does fall within previously accepted ranges as established by the reference data. Sampled Se levels were higher than the previous data, but no outward expression of Se toxicity symptoms were observed (NRC 2007; NASEM 2016). Herdt (1995) predicted that llamas with serum Se levels > 160 ng/mL, regardless of region raised, were adequate in their Se status which aligns with study sampled averages. Texas does not fall in a region known for high soil Se concentrations (Cech et al. 1984). This may imply that Se supplementation in captive operations exceeds cervid requirements or that white-tailed deer may tolerate higher serum Se levels than previously implied by reference data.

Ranch. Ranch location, management practices, and feeding programs show to play an important role in circulating micromineral blood serum levels of white-tailed does. Copper, Fe, Mn, and Zn levels were significantly higher at Ranch C. Further research of intake and mineral bioavailability of feedstuffs, soil, and water are needed to pinpoint the cause of these differences.

Ranch C had a large standard error for Fe attributed to females with exceptionally high levels. Although soil samples were not collected, Ranch C had red-tinged soil, characteristic of high Fe content, on the property (Dwevedi et al. 2017). This coupled with the available browse might explain the significantly higher serum Fe levels found in these does. Iron has an inhibitory effect on Co absorption, linking the low Co and high Fe levels observed at Ranch C (Reuber et al. 1994). Nevertheless, Co and Fe did not show a strong correlation when all does were considered (Table 6). This indicates that Ranches A and B did not reach Fe levels high enough to have an antagonist effect on Co.

Ranches A and B, which had higher serum vitamin A levels, both supplemented with alfalfa hay while Ranch C does consumed browse which is characteristically lower in vitamin A content during the winter breeding season (Sommer and West 1996). Weiss et al. (1995) reported that cattle fed high concentrate diets, similar to captive white-tailed does, had retinol disappearance of 80% due to lower ruminal pH. Additionally, vitamin A is highly sensitive to light and temperature making sampling difficult (Allwood 1982). These factors could explain the varying levels of vitamin A between ranches.

Age. Samples were not collected from does of all ages, 1-9 yrs, from all three ranches; this resulted in missing blocks of data. The lack of differences between age groups for Cu, Fe, Mn, vitamin A, and cholesterol may indicate that values can be applied to white-tailed does aged 1-9 yr. However, the significant differences between ages for Se, Zn, and vitamin E may indicate that diagnostic averages should be broken down by age as an all-inclusive value may not accurately represent white-tailed deer as they age. Gabryszuk and Klewiec (2002) reported a decrease in serum Se and vitamin E from 2 yr old to 3 yr old ewes. This trend was not observed in study sampled averages as maiden does were lower ($P < 0.01$) in serum Se and higher in serum vitamin E ($P < 0.01$) when compared to older age groups.

Interactions. Cobalt and Mo levels are dependent on the age of the doe as well as the location. No concrete pattern was established across ranch and age groups for Co or Mo. Further research with a more pointed focus is required to determine the impacts of locations, management, and age on Co and Mo.

Pregnancy Status. Females that failed to conceive on the day of sampling had lower circulating levels of plasma Zn compared to bred does. Tian and Diaz (2013) reported that Zn deficient diets fed 3-5 days prior to breeding are linked to a failure to develop a healthy oocyte and successfully conceive in mice. Additionally, Graham et al. (1995) linked low circulating Zn to higher prostoglandin F2 levels and subsequent reproductive failure in cattle. It should be noted that serum study averages for both open and bred females would be considered marginally Zn deficient when compared to the reference data.

Vitamin E was 0.41 µg/mL lower in open does in comparison to bred females. Vitamin E is necessary for the proper development of corpora lutea tissue resulting in higher pregnancy rates for vitamin E supplemented ewes (Vierk et al. 1998). Thus, vitamin E may serve as an area of greater concern in white-tailed deer supplementation going forward. Despite the importance of micronutrients in proper reproduction, statistical differences for the remaining micronutrients were not observed between open and bred females. This serves as an indication that established values are a representative base-line of healthy, reproductively sound does.

Future Research. Further research should evaluate the interaction between blood micromineral levels and levels present in the liver to further validate these two sampling methods as a diagnosis of mineral status. Pen studies using white-tailed deer, coupled with blood serum averages established in this study, would allow for a more accurate understanding of precise mineral supplementation requirements for captive raised white-tailed deer. Additionally, samples representing fawns, bucks, bred females, and lactating females would allow the data to be extrapolated to more classes of white-tailed deer.

Management Implications. Cervid management is growing in scope and intensity. It is paramount that animals are managed as safely and effectively as possible. The reported levels will contribute to the definition of micromineral and vitamin blood serum averages. Going forward, these values can serve as a stepping off point for more pointed nutritional trials. Long term, these blood micromineral and fat-soluble metabolite ranges can be used as a diagnostic tool for captive and free roaming white-tailed deer populations and in an effort to establish micronutrient requirements in white-tailed deer.

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