**Variation in Blood Selenium and Serum Vitamin E in Reindeer *(Rangifer tarandus tarandus)* Described by Location, Husbandry, and Season**

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**Abstract:** Reindeer (*Rangifer tarandus tarandus*) are important livestock for arctic and subarctic herders, including those in North America, but as climate change affects traditional herding practices, alternative methods of rearing (such as captive rearing) will likely become common. Proper nutrition is critical in livestock production, but there is minimal information available on circulating nutrient concentrations in reindeer, who are adapted to a unique climate. This study looks at 2 important antioxidants. Blood and serum were taken from female reindeer from three herds: a free range herd from the Seward Peninsula, Alaska (AK), during the summer, and two captive herds (one in Fairbanks, AK and one in Upstate New York (NY) during the summer and winter. Selenium (Se) and vitamin E concentrations were described stratified on season (when possible), location, and management practices (captive or free range).

 Mean (SD; N) summer Se were 4.17µmol/L (0.54; 14) [33µg/dL (4.3)], 2.4µmol/L (0.53; 30) [19µg/dL (4.2)], and 3.67µmol/L (0.67; 12) [29 µg/dL (5.3)] for the AK captive herd, the AK free-range herd, and the NY herd, respectively. The winter Se values for the two captive herds were: 4.05µmol/L (0.90; 31) [32µg/dL (7.1)] AK and 4.94µmol/L (0.70; 12) [39µg/dL (5.5)] NY. The lowest observed Se concentration, 0.53µmol/L (4.2µg/dL), was in summer in the AK free-range herd. Summer serum vitamin E concentrations were 4.37µmol/L (1.41; 29) [265µg/dL [(61)] AK Captive, 5.61µmol/L (1.21; 30) [242µg/dL (52)] AK free-range, and 7.74µmol/L (2.53; 12) [334µg/dL (109)] NY. The concentrations in winter were 5.27µmol/L (1.11; 31) [227µg/dL (48) AK captive, and 6.89µmol/L (2.48; 12) [297µg/dL (107)] NY. The lowest observed vitamin E concentration, 2.64µmol/L (114 µg/dL), was from the captive AK herd in the winter.

**Introduction:** Reindeer are a major livestock species in the arctic and subarctic. Reindeer herding was introduced to Native Alaskans in the late 1800s, and reindeer soon became valuable to traditional communities as a source of wholesome food and income. Reindeer-meat consumption is believed to contribute to the low prevalence of cardiac disease in European reindeer herders (Sampels et al. 2006). Reindeer meat is a healthy dietary choice because it is relatively low in fat, high in antioxidants such as Se and vitamin E, and high in nutritionally beneficial fatty acids (Rincker et al. 2006; Finstad et al. 2009). However, there has been an 80% decrease in the number of reindeer in Alaskan herds since the 1990s (Rattenbury et al. 2009).

If reindeer production is to meet the needs of native Alaskans, captive-herd management is a sustainable option. Captive and pasture reindeer management, however, is currently not considered cost effective (Rattenbury et al., 2009). Despite reduced transportation cost (e.g. snowmobiles) and greater carcass weight compared to free-range herding, there is increased feed cost (Furberg et al. 2011). Captive rearing requires more intensive nutritional management, demonstrated by captive-herd practices in the state of NY (where reindeer are raised for commercial services such as holiday promotions) and in Fairbanks, AK. Reindeer are adapted to the marked seasonal changes in forage availability and quality in the arctic and subarctic (Borch-Iohnsen et al. 1996; Aastrup et al. 2000; Finstad 2008). Even when fed in captivity, feed intake decreases during the winter (Mesteig et al. 2000). Reindeer have evolved to survive in the tundra ecosystems consuming natural forage, but as they move to captivity and manufactured rations, more attention to baseline nutritional information is needed. Antioxidants like vitamin E and Se are critical for health and reproduction in livestock and deficiency in either one is associated with nutritional muscular dystrophy and fetal and neonatal deaths (Valberg 2012), but although a few studies have found reindeer meat to be a good source of vitamin E and selenium, there is relatively little information on circulating antioxidants in live reindeer (Hassan et al. 2012a, Hassan et al. 2012b).

This focus of this study is to describe circulating Se and vitamin E concentrations in three reindeer herds that are located in two different states (temperate NY and subarctic AK) and have two different husbandry systems (captive and free-range); we show the descriptions separately for summer and winter. There is no standardized diet for reindeer at this time, and the captive herds consumed different diets. Although data on feed intake were lacking, each herd had excellent herd production records and no evidence of nutritional imbalance.

**Materials and Methods:** We sampled three reindeer herds, including a free-range herd in Seward, AK of 800 to1000 head. The other two herds were captive herds: one each in Fairbanks, AK (approximately 90 head, pasture access) and in Upstate NY (approximately 20 head, limited pasture access). The herds in AK are supervised by the Reindeer Research Project and data are collected on ear-tagged individuals. The captive AK reindeer were at the University of Alaska Agricultural and Forestry Experiment Station (UAF-AFES). These animals consume 3.1% of body weight in dry matter (dm)/day during summer and 1.5% of body weight in dm/day during winter. They are fed a milled ration *ad lib* every day; they are on pasture (Kentucky Nugget Bluegrass) in summer and fed Wheatgrass haylage in winter (Finstad 2015). The herd in NY is not a research herd, but it has regular veterinary supervision. A 20% protein feed is milled for the NY herd.

 Due to animal dispersion over winter and field conditions, it was not possible to collect multiple samples from the free-range herd, but samples were collected from the same individuals when possible in the captive herds. Blood and serum were collected via routine jugular venipuncture from mature (≥1 year old) female reindeer that appeared healthy based on behavior and physical-exam findings. Blood samples were collected into two types of 10-mL Vacutainer® tubes: one with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and one without an anticoagulant. Whole blood was refrigerated after collection and packed on ice for transport to the Animal Health Diagnostic Center (AHDC) for analysis. Blood collected into a serum tube was always kept in low-light conditions, allowed to clot, then centrifuged to separate the serum, which was transferred into a clean tube without anticoagulant and stored frozen (approximately -10° to -20°C; blanketed and protected from bright light), and shipped to the AHDC for analysis. Blood tubes were stored in the refrigerator and serum tubes in the freezer at the AHDC until analysis was performed. Collections took place in 2013 on June 28 for the free-range herd, February 4 and July 9 for the captive AK herd, and January 20 and June 25 for the captive NY herd.

Selenium concentrations in homogenous whole blood were determined using Atomic Absorption Graphite Furnace (AA-GF) with longitudinal Zeeman-effect background corrector. Whole blood was diluted 10-fold with a matrix modifier (a mixed solution (500:400 ratio) of palladium chloride and nickel(ous) nitrate solution, and ammonium phosphate and magnesium nitrate solution) and a 10µL aliquot was dispensed by the autosampler into the graphite furnace for analysis. (Palladium Chloride Solution is prepared by adding 1.667g PdCl2 to 1L of Nano-pure, deionized water that contains 2% HNO3. Palladium chloride and nickel(ous) nitrate solution is prepared by adding 12.5g Ni (NO3)2\*6H2O and 1mL Triton X qs to 550mL palladium chloride solution to a volume of 1L. Ammonium phosphate and magnesium nitrate solution is Nanopure®, deionized, or double distilled water that contains 0.2%NH4H2PO4 and 0.05% Mg(NO)3 in 1% HNO3 and 0.1% Triton X. Calibration curves were established for each run using the calibration standards. A 1.26µmol/L (10µg/mL) Se, International Organization for Standards (ISO)-approved reference stock solution, was used to prepare the Intermediate Standard Solutions with matrix modifier. Calibration standard concentrations are 0.32µmol/L (2.50µg/dL), 1.23 µmol/L, 3.17µmol/L (25.00µg/dL), and 6.33µmol/L (50.00µg/dL).

Vitamin E concentrations as alpha-tocopherol were determined using a high-pressure liquid chromatograph (HPLC) and fluorescence spectrometer with a Agilent Eclipse XDB-C18, 5-micron particle size, 15cm x 4.6mm internal diameter with the following specifications: column temperature of 40°C, flow rate of 1.4mL/minute, excitation wavelength 291nm (10nm slit width), and emission wavelength 330nm (10nm slit width). Retention time for alpha-tocopherol is approximately 10.5minutes. Analysis is performed in subdued light. One mL serum was placed in a 15mL centrifuge tube and weighed accurately. One mL ethanol was added to the sample and vortexed for 10 seconds. Two mL hexane was added and vortexed for 1 minute. Samples were placed in a rotating shaker for 5 minutes, then left undisturbed for layers to separate for 10 minutes. Hexane was transferred to a disposable glass culture tube with a transfer pipet. Extraction was repeated with one mL hexane, with samples centrifuged at 2000 rpm for 5 minutes after removal from rotating shaker. Hexane collection was repeated and the pooled hexane extracts evaporated to dryness at 30 to 40°C under nitrogen. 600µL of solution containing 684mL acetonitrile + 220mL tetrahydrofuran + 70mL methanol + 30mL 1% ammonium acetate solution (W/V) was added to each tube and vortexed for approximately 1 minute. The resulting solution was transferred to an amber glass autosampler vial and capped for injection into the HPLC system. Aliquots of 30µL of each working standard solution and each sample solution were injected. The peak area of the eluted peak for alpha-tocopherol for all standard solutions and sample solutions was recorded. Alpha-tocopherol standards were made from approximately 97% purity alpha-tocopherol (obtain Certificate of Analysis with actual percent purity from supplier). The actual percent purity of the alpha-tocopherol was verified by UV spectrophotometry at 285nm using the extinction coefficient for alpha-tocopherol. The stock standard solution for alpha-tocopherol was approximately 20.8µmol/L (899.1µg/mL) alpha-tocopherol, which was made by weighing approximately 0.045g of approximately 97% purity alpha-tocopherol into a 50mL volumetric flask and taking it to volume with ethanol (Hess et al. 1991). The alpha-tocopherol content of each sample was calculated by comparing the sample solution peak area to the standard curve.

The free-range herd in AK consumed forage only, whereas the captive herds were given formulated diets (Table 1) in addition to limited pasture access. Formulated feed for the captive AK herd was analyzed for Se at the AHDC and for vitamin E at the Michigan Animal Health Diagnostic Laboratory. Formulated feed for the NY herd was analyzed for Se and vitamin E at Dairy One Inc. Forage Testing Laboratory (Ithaca NY).

Statistix® 10 software was used for statistical analysis (Analytical Software, Talahassee, FL). Data subsets first were analyzed using Shapiro-Wilk Normality tests to determine whether the data were Gaussian.

**Results:** The Alaskan supplemented-feed nutrient concentrations (in diets of captive reindeer on an as-fed basis) were 4.81 µmol/kg (0.38 mg/kg) for Se, which was higher by inspection than the 0 to 3.80 µmol/kg (0 to 0.3 mg/kg) label values (Table 1), and 18 IU/kg for vitamin E. New York supplemented-feed nutrient concentrations, also on an as-fed basis, were 11.0 µmol/kg (0.87 mg/kg) Se and 553 IU/kg vitamin E. Several data points are missing because some individual animals were lost to follow-up and some analytical results were lost due to the unanticipated effect of reindeer blood, which is highly viscous, on the autosampler (Feist & White 1989).

 All data appeared to be Gaussian after testing for normality, so data are described by the mean and SD, as well as by the minimum and maximum. Table 2 lists descriptions for both summer and winter for all herds combined and for each herd. We noted some large variations in the observed serum concentrations of SE and vitamin E within the apparently healthy female reindeer.

**Discussion:** Changes in nutrient access and availability for free range animals affect biochemical parameters (Miller et al. 2013). Free-range reindeer are be expected to experience extreme changes in forage type and availability over the period of a year, and climate change is expected to enhance this effect (Nieminen & Heiskari 1989; Weladji & Holand 2003; Finstad 2008; Bartsch et al. 2010). Furthermore, even captive reindeer decrease their feed intake during the winter (Mesteig et al. 2000). Unfortunately, traditional herding practices, which involve allowing animals to disperse over winter, and extreme climatic conditions made collection of winter samples from free range reindeer in the field impractical.

Blood Se ranges and means for the three herds and two seasons are listed in Table 2. Only females were sampled in this study, but a previous study of free range reindeer found a significant difference in blood Se concentrations between males and females, attributed to different foraging patterns, though it did not separately list results for males and females (Miller et al. 2013). That study collected samples in November and found a mean blood Se concentration of 0.003µmol/g (0.21μg/g) (n=17, SD = 0.001µmol/g [0.01µg/g]), which is approximately 2.66µmol/L (21μg/dL), similar to the summer values we found in free-range reindeer: 2.42µmol/L, range 0.53 to 3.29µmol/L (19.1μg/dL range 4.2 to 26.0μg/dL). Another study of found similar blood Se concentrations, but there was variability based on the geographic range of the herds (Finstad 2008). No blood Se values for captive reindeer were discovered in the available literature, but the mean values from the captive reindeer in our study (3.62 to 4.88µmol/L [28.6 to 38.5 μg/dL] across herds and seasons) appeared similar (by inspection) to the mean value of 4.67µmol/L (37.0μg/dL) for captive white-tailed deer (*Odocoileus virginianus*). The mean value for captive antelope (*Antilocapra americana*) seemed higher: 6.71µmol/L (53.0 μg/dL) (Clemens et al. 1987).

 Selenium requirements for domestic animals range from 1.27 to 3.80µmol/g (0.10 to 0.30 mg/kg) in the total diet (Klasing et al. 2005). Dietary Se and vitamin E concentrations are expected to influence both the circulating Se and vitamin E concentrations and the concentrations of these nutrients in animal products. Reindeer from Finland, Norway, and Sweden had higher tissue Se and vitamin E concentrations than caribou (*Rangifer tarandus caribou)* in Canada (Hassan et al. 2012b). Other studies have found variation in Se concentrations in meat and liver from reindeer based on geographic location (Vikoren et al. 2011; Hassan et al. 2012b). Se uptake in plants is related to soil Se content and availability, climate, and plant factors (Ullrey 1988; Mayland et al. 1991). The unsupplemented free-range herd in this study was in the Kenai Peninsula Borough of Alaska, where the forage had adequate Se for livestock (Finstad 2008). Data from sediments in the North Star Borough, the location of one of the captive-with-pasture herds in this study, found Se concentrations up to 20.2µmol/kg (1.60mg/kg), but most samples contained < 12.7µmol/kg (1mg/kg). The NY herd was located in Steuben County, where soil Se concentrations range from 1.27 to 4.43µmol/kg (0.10 to 0.35mg/kg) with a mean of 1.90µmol/L (0.15mg/kg, http://mrdata.usgs.gov/geochem/doc/averages/se/usa.html). Forage plants from Se-deficient areas such as these usually contain <0.6µmol/kg [0.05mg/kg] Se and thus can be considered a negligible source of dietary Se. The major source of Se for the captive herds was the supplemented concentrate feeds, containing 4.81µmol/kg (0.38mg/kg, based on laboratory analysis) and 11.01µmol/kg (0.87mg/kg) Se for Alaska and New York, respectively.

Fresh forage is a major contributor of vitamin E (as alpha-tocopherol) to ruminant diets, but vitamin E is lost during drying and storage (Ballet et al. 2000). The Alaska captive herd’s feed contained 18 IU vitamin E/kg, whereas the New York herd’s ration contained 553 IU vitamin E/kg. The by-inspection herd-mean differences were not proportional to the difference in supplementation. All reindeer in this study had some access to forage year-round, although the captive herds (particularly in NY) were limited by pasture size. The ratio of pasture land per individual animal in NY versus AK and the types of forage plants available in the different areas likely affect vitamin E intake.

A previous study found clinical vitamin E deficiency in captive moose in central AK, with a mean serum vitamin E concentration < 0.18µmol/L (8μg/dL) compared to 6.5µmol/L (280μg/dL) for wild moose (Stephenson et al. 2001). The moose were maintained on a pelleted ration containing 5 IU vitamin E/kg for 9 months and exhibited reproductive failure and calf losses. Plasma vitamin E concentrations in health have been reported for a variety of captive species of deer: 9.81µmol/L (423μg/dL) in Axis deer (*Axis axis)*; 6.61µmol/L (285 μg/dL) in Eld’s deer (*Panolia eldii*); and 4.06µmol/L (175μg/dL) in Sika deer (*Cervus nippon*). Values of 0.70µmol/L (30μg/dL), 0.70µmol/L , and 2.09µmol/L (90 μg/dL), respectively, were associated with clinical deficiency in these deer species (Dierenfeld 1994). The circulating vitamin E concentrations in our study appeared to be similar to those in Eld’s (*Panolia eldii*) and Sika deer (*Cervus nippon*) and higher (by inspection) than those associated with deficiency.

Further studies are needed to determine whether circulating vitamin E and Se concentrations correlate with those found in the meat and to test whether serum concentrations of Se and vitamin E are associated with factors such as location of the pasture, season, and husbandry system.

**Conclusions:** In adult female reindeer that were apparently healthy, we found circulation concentrations of Se as low as 0.53 µmol/L (4.2mg/dL) and of vitamin E as low as 2.64µmol/L (114mg/dL). Herd mean values across seasons for Se ranged from 2.42 to 4.88µmol/L (19.1 to 38.5mg/dL). Herd mean values across seasons for vitamin E ranged from 5.27 to 6.89µmol/L (227 to 334mg/dL).

**Acknowledgement:** The authors thank the Cervid Livestock Foundation for their generous support of this project.

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| --- | --- | --- |
|  | Alaska | New York |
| Analysis | 16% crude protein | 20% crude protein |
|  | Soybean meal | Whitefish meal | Soybean meal |
| NDF (%) | 23.58 | 25.13 | 33.1 |
| ADF (%) | 9.52 | 10 | 19.3 |
| Crude Protein (%) | 16.13 | 16.13 | 20.8 |
| Phosphorus (%) | 0.41 | 0.42 | 1.19 |
| Potassium (%) | 0.79 | 0.78 | 1.38 |
| Calcium (%) | 0.73 | 0.64 | 1.61 |
| Magnesium (%) | 0.17 | 0.15 | 0.50 |
| Sulfur (%) | 0.17 | 0.17 | 0.87 |
| Sodium (mg/kg) | 1214  | 1020 | 1214  |
| Copper (mg/kg) | 18.6 | 13 | 45 |
| Iron (mg/kg) | 168 | 184 | 533 |
| Manganese (mg/kg) | 123 | 115 | 171 |
| Molybdenum (mg/kg) | 0.6 | 0.9 | 1.1 |
| Se (mg/kg) | 0.03 | 0 | 0.87 |
| Zinc (mg/kg) | 138 | 129 | 279 |

Table 1: Nutritional profile of two of the feed mixes fed to the Alaska Reindeer Research Program captive deer and for the New York herd, on an as-fed basis.

|  |  |  |
| --- | --- | --- |
|  |  |  |
|  | **All Reindeer** | **AK Captive** | **AK Free Range**  | **NY Captive** |
| Se Summer | 3.11µmol/L, SD=0.95(24.6μg/dL,n=56, SD=7.5)0.53, 5.19µmol/L(4.2, 41.0μg/dL) | 4.15µmol/La, SD=0.54(32.8μg/dL,n=14, SD=4.3)3.28, 5.21µmol/L(25.9, 41.0μg/dL) | 2.42µmol/Lb, SD=0.53(19.1μg/dL, n=30, SD=4.2)0.53, 3.29µmol/L(4.2, 26.0μg/dL) | 3.62µmol/La, SD=0.67(28.6μg/dL,n=12, SD=5.3)2.53, 5.18µmol/L(20.0, 40.9μg/dL) |
| Se Winter | 4.28µmol/L, SD=0.92(33.8μg/dL, n=43, SD=7.3)2.51, 6.28µmol/L(19.8, 49.6μg/dL) | 4.05µmol/Ld, S=0.90(32.0μg/dL,n=31, SD=7.1)2.51, 5.93µmol/L(19.8, 46.8μg/dL) | No data | 4.88µmol/Lc, SD=0.70(38.5μg/dL,n=12, SD=5.5)4.07, 6.28µmol/L(32.1, 49.6μg/dL) |
|  |  |  |  |  |
| Vit E Summer | 6.19µmol/L, SD=1.72; n=71(267μg/dL, SD=74.3)3.39, 13.15µmol/L(146, 567μg/dL) | 6.19µmol/Lf, SD=1.42; n=29(265μg/dL,SD=61.0)3.94, 9.72µmol/L(170, 419μg/dL) | 5.61µmol/Lf, SD=1.21; n=30(242μg/dL,SD=52.0)3.38, 8.42µmol/L(146, 363μg/dL) | 7.75µmol/Le, SD=2.53; n=12(334μg/dL,SD=109)3.80, 13.2µmol/L(164, 567μg/dL) |
| Vit E Winter  | 5.71µmol/L, SD=1.74; n=43(246μg/dL,SD=75.2)2.64, 12.3µmol/L(114 ,531μg/dL) | 5.27µmol/Lg, SD=1.12; n=31(227μg/dL,SD=48.1)2.64, 7.66µmol/L(114, 330μg/dL) | No data | 6.89µmol/Lg, SD=2.48; n=12(297μg/dL,SD=107)4.18, 12.3µmol/L(180, 531μg/dL) |
|  |  |  |  |  |

Table 2: Blood Se and vitamin E concentrations mean concentration, n, SD, and minimum and maximum. collected in summer and winter from reindeer from captive herds in Alaska and New York and a free range herd in Alaska.

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