

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-ranging 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). Herd mean values across seasons for Se ranged from 2.42 to 4.88 $\mu\text{mol/L}$. Herd mean values across seasons for vitamin E ranged from 5.27 to 6.89 $\mu\text{mol/L}$.

Key words: Selenium; vitamin E; alpha-tocopherol; antioxidant.

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Introduction

Reindeer (*Rangifer tarandus tarandus*) is 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 New York (NY) (where reindeer are raised for commercial services such as holiday promotions) and in Fairbanks, Alaska (AK). Reindeer are adapted to the marked seasonal changes in forage availability and quality in the arctic and subarctic (Borch-Johnsen *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-ranging); 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-ranging herd in Seward, AK of 800 to 1000 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 Program 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). 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-ranging 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-ranging 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 $10\mu\text{L}$ aliquot was dispensed by the autosampler into the graphite furnace for analysis. Palladium Chloride Solution is prepared by adding 1.667g PdCl_2 to 1L of Nanopure, deionized water that contains 2% HNO_3 . Palladium chloride and nickel(ous) nitrate solution is prepared by adding 12.5g $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 1mL Triton X qs to 550 mL palladium chloride solution to a volume of 1 L. Ammonium phosphate and magnesium nitrate solution is Nanopure[®], deionized, or double distilled water that contains 0.2% $\text{NH}_4\text{H}_2\text{PO}_4$ and 0.05% $\text{Mg}(\text{NO}_3)_2$ in 1% HNO_3 and 0.1% Triton X. Calibration curves were established for each run using the calibration standards. A $126\mu\text{mol/L}$ Se, International Organization for Standards (ISO)-approved reference stock solution, was used to prepare the Intermediate Standard Solutions. Calibration standard concentrations are $0.32\mu\text{mol/L}$, $1.23\mu\text{mol/L}$, $3.17\mu\text{mol/L}$, and $6.33\mu\text{mol/L}$.

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, 15 cm x 4.6 mm internal diameter with the following specifications: column temperature of 40°C , flow rate of 1.4 mL/minute, excitation wavelength 291 nm (10 nm slit width), and emission wavelength 330 nm (10 nm slit width). Retention time for alpha-tocopherol is approximately 10.5 minutes. Analysis is performed in subdued light. One mL serum was placed in a 15 mL 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. $600\mu\text{L}$ of solution containing 684 mL acetonitrile + 220 mL tetrahydrofuran + 70 mL methanol + 30 mL 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 $30\mu\text{L}$ 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 285 nm using the extinction coefficient for alpha-tocopherol. The stock standard solution was approximately $2080\mu\text{mol/L}$ alpha-tocopherol, which was made

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.

Analysis	Alaska		New York
	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

by weighing approximately 0.045 g of approximately 97% purity alpha-tocopherol into a 50 mL volumetric flask and taking it to volume with ethanol (Hess *et al.*, 1991).

The free-ranging 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, Tallahassee, FL). Data subsets first were analyzed using Shapiro-Wilk Normality tests to determine whether the data were Gaussian. We also looked at mean relative to median; location of the median within the range; the coefficients of variation for the seasonal values; and those same values

by herd in box-and-whiskers plots. Data then were described using the mean and standard deviation (SD), with minima and maxima to provide information on extreme values observed in healthy animals.

Results

The Alaskan supplemented-feed nutrient concentrations (in diets of captive reindeer on an as-fed basis) were 4.81 $\mu\text{mol/kg}$ for Se, which was higher by inspection than the 0 to 0.38 $\mu\text{mol/kg}$ 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 $\mu\text{mol/kg}$ 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 test-

ing 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 ranging animals affect biochemical parameters (Miller *et al.*, 2013). Free-ranging reindeer are 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) particularly the protein and mineral contents, of ground lichens (*Cladonia* spp.). Furthermore, even captive reindeer reduce their feed intake during the winter (Mesteig *et al.*, 2000) at least in part, a consequence of seasonal fluctuation in voluntary-food intake. Heart rate and daily dry matter voluntary-food intake (DDMVFI). Unfortunately, traditional herding practices, which involve allowing animals to disperse over winter, and extreme climatic conditions made collection of winter samples from free-ranging rein-

Table 2. Blood Se and vitamin E concentrations (mean values \pm standard deviation, SD), number of analyzed samples (*n*), and minimum and maximum values. Samples collected in summer and winter from reindeer from captive herds in Alaska (AK) and New York (NY) and a free-ranging herd in Alaska.

	All Reindeer	AK Captive	AK Free-ranging	NY Captive
Se ($\mu\text{mol/L}$)				
<u>Summer</u>	3.11 \pm 0.95 (<i>n</i> = 56)	4.15 \pm 0.54 (<i>n</i> = 14)	2.42 \pm 0.53 (<i>n</i> = 30)	3.62 \pm 0.67 (<i>n</i> = 12)
<i>Min</i>	0.53	3.28	0.53	2.53
<i>Max</i>	5.19	5.21	3.29	5.18
<u>Winter</u>	4.28 \pm 0.92 (<i>n</i> = 43)	4.05 \pm 0.90 (<i>n</i> = 31)	No data	4.88 \pm 0.70 (<i>n</i> = 12)
<i>Min</i>	2.51	2.51		4.07
<i>Max</i>	6.28	5.93		6.28
Vit E ($\mu\text{mol/L}$)				
<u>Summer</u>	6.19 \pm 1.72 (<i>n</i> = 71)	6.15 \pm 1.42 (<i>n</i> = 29)	5.61 \pm 1.21 (<i>n</i> = 30)	7.75 \pm 2.53 (<i>n</i> = 12)
<i>Min</i>	3.39	3.94	3.38	3.80
<i>Max</i>	13.15	9.72	8.42	13.2
<u>Winter</u>	5.71 \pm 1.74 (<i>n</i> = 43)	5.27 \pm 1.12 (<i>n</i> = 31)	No data	6.89 \pm 2.48 (<i>n</i> = 12)
<i>Min</i>	2.64	2.64		4.18
<i>Max</i>	12.3	7.66		12.3

deer in the field impractical. We were therefore unable to reveal any effect of season on free-ranging reindeer.

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-ranging reindeer found a significant difference in blood Se concentrations between males and females, attributed to different foraging patterns (Miller *et al.*, 2013). That study collected samples in November and found a mean blood Se concentration of 0.0028 $\mu\text{mol/g}$ in females ($n=11$) and 0.0024 $\mu\text{mol/g}$ in males ($n=6$), or approximately 2.94 and 2.52 $\mu\text{mol/L}$, similar to the summer values we found in free-ranging reindeer (2.42 $\mu\text{mol/L}$). 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 4.88 $\mu\text{mol/L}$ across herds and seasons) appeared similar (by inspection) to the mean value of 4.67 $\mu\text{mol/L}$ for captive white-tailed deer (*Odocoileus virginianus*). The mean value for captive antelope (*Antilocapra americana*) seemed higher: 6.71 $\mu\text{mol/L}$ (Clemens *et al.*, 1987).

Selenium requirements for domestic animals range from 1.27 to 3.80 $\mu\text{mol/g}$ 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 woodland 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 (Ulrey 1988; Mayland *et al.*, 1991). The unsupplemented free-ranging 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 20.2 $\mu\text{mol/kg}$, but most samples contained < 12.7 $\mu\text{mol/kg}$. The NY herd was located in Steuben County, where soil Se concentrations range from 1.27 to 4.43 $\mu\text{mol/kg}$ with a mean of 1.90 $\mu\text{mol/L}$ (<http://mrdata.usgs.gov/geochem/doc/averages/se/usa.html>). Forage plants from Se-deficient areas such as these usually contain < 0.6 $\mu\text{mol/kg}$ 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 4.81 $\mu\text{mol/kg}$ (based on laboratory analysis) and 11.01 $\mu\text{mol/kg}$ 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 < 0.18 $\mu\text{mol/L}$ compared to 6.5 $\mu\text{mol/L}$ 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 exhib-

ited reproductive failure and calf losses. Plasma vitamin E concentrations in health have been reported for a variety of captive species of deer: 9.81 $\mu\text{mol/L}$ in Axis deer (*Axis axis*); 6.61 $\mu\text{mol/L}$ in Eld's deer (*Panolia eldii*); and 4.06 $\mu\text{mol/L}$ in Sika deer (*Cervus nippon*). Values of 0.70 $\mu\text{mol/L}$, 0.70 $\mu\text{mol/L}$, and 2.09 $\mu\text{mol/L}$, 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 and Sika deer 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 $\mu\text{mol/L}$ and of vitamin E as low as 2.64 $\mu\text{mol/L}$. Herd mean values across seasons for Se ranged from 2.42 to 4.88 $\mu\text{mol/L}$. Herd mean values across seasons for vitamin E ranged from 5.27 to 7.75 $\mu\text{mol/L}$.

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