VITAMIN E, SELENIUM, AND REPRODUCTIVE LOSSES IN ALASKAN MOOSE

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ABSTRACT: A severe vitamin E deficiency was observed in a captive moose (*Alces alces*) population that was maintained on a pelleted ration during 9 months per year. During 1998 only 10 of 17 calves identified in utero using ultrasonography at the Moose Research Center (MRC), Alaska, were born alive. An additional 3 calves exhibited posterior lameness within 3 weeks following birth and 2 of the 3 subsequently died. These symptoms have been previously associated with white muscle disease. White muscle disease results from both vitamin E and selenium deficiencies. While whole blood and liver selenium levels in 3 animals with white muscle disease were above recommended levels, serum vitamin E (α-tocopherol) levels for MRC calves were lower than levels observed in free-ranging neonatal calves in interior Alaska (Tanana Flats). Furthermore, mean serum vitamin E levels in adult cows during March at the MRC (0.08 μg/ml) were alarmingly lower than free-ranging Tanana Flats moose (2.8 μg/ml). We observed vitamin E deficiencies in animals fed diets with 5 IU/kg feed. Our data suggest that clinical symptoms of vitamin E deficiencies in adult moose may be difficult to detect, unless animals are reproducing. Following supplementation of vitamin E to 220 IU/kg in our pelleted ration during 1999, we observed no abortions and only 1 cow had still-born twin calves, but this was attributed to dystocia. Indeed, during 1999 only 2 of 16 calves identified in utero died of nonpredation causes. Although a vitamin E deficiency in free-ranging moose is unlikely, low selenium levels have been observed in free-ranging ungulate populations. Mean whole blood selenium levels in Tanana Flats moose (0.12 μg/g) were significantly lower than MRC adult cows (0.16 μg/g) fed a supplemented diet. More importantly, 8 of 10 animals from the Tanana Flats had selenium levels $\leq$ 0.085 μg/g and were below recommended levels for domestic cattle. Given the lack of data on soil selenium levels in Alaska, deficiency-related neonatal losses may occur that are attributed to other causes of mortality. It will be difficult to quantify in utero and neonatal calf losses resulting from selenium and vitamin E deficiencies if blood or tissue samples from study locations are not examined.

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Essential dietary nutrients for proper growth and survival include numerous vitamins and minerals. Vitamin E and selenium protect biological membranes from oxidative degeneration and deficiencies in them result in the breakdown of tissues. Vitamin E functions largely as a lipid antioxidant by protecting membranes in most cells from oxidative degradation (Combs 1992). Selenium is an essential constituent of the enzyme glutathione peroxidase that destroys peroxides before damage to lipid membranes

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occurs (McDowell 1992).

Free-ranging ungulates obtain vitamin E by consuming plants that synthesize it, and in particular the green parts of plants that contain α-tocopherol. A labile pool of vitamin E occurs in tissues such as plasma and liver; a fixed pool for long-term storage occurs in adipose tissue (Combs 1992). Selenium concentrations in natural forages are determined by levels of available selenium in the soil and are generally low in the northeast, southeast, and northwest United States, as well as areas adjoining the Great Lakes. Storage of selenium occurs in the kidney, liver, and other glandular tissue (McDowell 1992).

Dierenfeld (1989) reviewed vitamin E deficiencies in reptiles, birds, and ungulates housed in zoos. Deficiencies of Vitamin E may be manifested, among other means, through various forms of reproductive failure in mammals. In particular, vitamin E deficiencies result in fetal death and abortion, as well as white muscle disease (WMD) in neonates. Similarly, selenium deficient young may exhibit WMD whereas retained placenta are uniquely a consequence of females suffering from selenium deficiencies (Julien et al. 1976, Hurley and Doane 1989).

We describe a vitamin E deficiency in a population of captive moose in Alaska. In addition, we sampled a free-ranging moose population in Alaska and quantified vitamin E and selenium levels and compared these to levels in our captive population.

STUDY AREA AND METHODS

Research on captive moose occurred at the Moose Research Center located on the Kenai Peninsula, Alaska (60°N, 150°W). Samples from free-ranging moose were collected on the Tanana Flats and in the foothills of the Alaska Range (64°N, 147°W); this area was described in detail by Keech et al. (2000) and Gasaway et al. (1983).

Moose Research Center

We conducted feeding trials with captive moose as part of a broader study on the effects of nutrition on body condition and reproductive performance (Stephenson et al. 1999). Beginning in November 1997, 10 adult female moose were fed in trials that included a high digestibility pelleted moose feed (Schwartz et al. 1985) and a lower digestibility ration (Stephenson et al. 1999). Both rations contained 5 IU vitamin E/kg. During October 1997 – May 1998, rations were offered ad libitum. Animals, confined together in a 4-ha fenced enclosure, accessed feed, using individual-specific feed gates (American Calan, Inc., Northwood, New Hampshire, USA) developed for controlled-access feeding trials. The system utilizes a feed container, accessible only through a neck slot controlled by a 24-volt electronically locking gate that is unlocked by an individual-specific sensing "key" collar worn by the animal (Mazaika et al. 1988). Known amounts of feed were offered and orts were collected daily to permit calculation of daily energy and protein intake for each animal. During the October-May trial period, diets of trial moose consisted almost entirely of pelleted feed except for limited amounts of spruce (*Picea* spp.). During the remainder of the year (June-September), animals browsed entirely on native shrubs, grasses, and forbs. A second feeding trial was conducted during 16 November 1998 – 30 April 1999 but all feeds were supplemented with 220 IU vitamin E/kg.

During both trial years, moose were immobilized during September, November, January, March, and April using carfentanil citrate/xylazine hydrochloride and reversed with naltrexone/tolazoline. Portable, real-time ultrasound was used to diagnose initial reproductive condition during November 1997 and 1998. We transrectally scanned

cows using an Aloka model 500 ultrasound device (Aloka, Inc., Wallingford, Connecticut, USA) with a 5 MHz 8 cm linear-array transducer to detect the presence, viability, and number of fetuses (Stephenson et al. 1995). Serum was collected during all immobilizations for determination of pregnancy-specific protein B (PSPB) levels (Huang et al. 1999, 2000). In addition, moose were weighed in September and weekly during feeding trials.

Newborn calves located by ground surveillance of cows were captured by hand. Calves were handled during 12 – 48 hours postpartum. Captured calves were equipped with expandable radio collars and numbered eartags. Sex, body mass, total body length, and hind foot length were recorded at capture. Serum was collected and evaluated for determination of Vitamin E (α-tocopherol). In addition, when available postmortem from animals that exhibited white muscle disease, we submitted whole blood or liver samples for determination of selenium.

Assays of serum vitamin E and whole blood selenium were conducted by the Washington Animal Disease and Diagnostic Laboratory, Pullman, Washington, USA. Vitamin E (α-tocopherol) was determined by high performance liquid chromatography. Total selenium was quantified by ICP atomic emission. A *Z*-test was used to test for a difference in the proportion of calves surviving between 1998 and 1999. *t*-tests were used to test for differences in vitamin E and selenium between the MRC and the Tanana Flats. Paired *t*-tests were used to test for differences in cow vitamin E levels between March 1998 and 1999. Analyses were conducted using program SAS (SAS Institute, Cary, North Carolina, USA) and program SYSTAT (SPSS, Inc., Chicago, Illinois, USA).

Tanana Flats

Adult female moose were immobilized during March 1996 and 1997 on the Tanana Flats (Keech et al. 2000). A mixture of carfentanil citrate/xylazine hydrochloride was administered by dart rifle (Palmer Cap-Chur Equipment, Douglasville, Georgia, USA) during helicopter pursuit. Blood was collected by jugular venipunture and serum and whole blood were stored at –20°C. Cows were collared with frequency-specific VHF transmitters.

Neonatal calves of radio-collared cows were located and captured within 48 hours postpartum using a helicopter (Keech et al. 2000). Calves were weighed, blood was collected by jugular venipunture, and serum was stored at –20°C.

RESULTS

Calves

During 1998 only 10 of 17 calves identified in utero using ultrasonography at the Moose Research Center (MRC), Alaska, were born alive; 4 were aborted and 3 were stillbirths. PSPB profiles indicated that of the 4 cows that aborted, 1 aborted early in gestation (PSPB remained < 50 ng/ml) and the remaining 3 were late term abortions (PSPB > 500 ng/ml between 5 and 6 months postconception). Furthermore, an additional 3 calves exhibited symptoms of white muscle disease (e.g., posterior lameness) within 3 weeks following birth and 2 subsequently died. Necropsy of one of these calves revealed multifocal, severe, myofiber degeneration and fibrosis during histological inspection of skeletal muscle. This pathology is indicative of white muscle disease. Hence during 1998, 59% of fetuses or calves died from non-predation mortality. By contrast during 1999, 2 of 16 (12.5%) calves died from non-predation sources and both of these were due to dystocia during birth in the same cow. Consequently, substantially fewer calves died ($Z = 16.61$, $P < 0.01$)

following supplementation with vitamin E during 1999.

Whole blood and liver selenium levels were 0.14 and 0.16 μg/g and 1.8 μg/g, respectively in 3 calves with white muscle disease that were sampled; both are above recommended levels (McDowell 1992). In contrast, mean serum vitamin E (α-tocopherol) level was 0.63 μg/ml (range 0.53 - 0.8 μg/ml) for MRC calves which was lower ($t = 6.3$, df = 4, $P = 0.003$) than levels observed in free-ranging neonatal calves (2.36 μg/ml, SE = 0.27) in interior Alaska (Tanana Flats).

Adult Females

Mean serum vitamin E levels in adult cows during March 1998 at the MRC (0.08 μg/ml, SE = 0.02) were alarmingly lower (t = 9.8, df = 4, P = 0.0006) than in free-ranging Tanana Flats moose (2.8 μg/ml, SE = 0.28). However, following increased supplementation of dietary vitamin E, mean serum vitamin E levels in paired samples of MRC cows during 1999 had increased (t = -7.08, df = 6, $P < 0.001$) to 0.78 μg/ml (SE = 0.09).

Mean whole blood selenium levels in Tanana Flats moose (0.12 μg/g, SE = 0.013) were lower ($t = 2.5$, df = 22, $P = 0.02$) than MRC adult cows (0.16 μg/g, SE = 0.004), fed a supplemented diet. In addition, 8 of 20 (40%) animals from the Tanana Flats had selenium levels ≤ 0.085 μg/g.

DISCUSSION

McDowell (1992) described 2 clinical patterns in neonatal ruminants of white muscle disease, a primary symptom of vitamin E and selenium deficiencies, and we observed both at the MRC. One is a congenital form of muscular dystrophy in which young are stillborn or die within a few days postpartum. Our high incidence of late term abortions/still births is likely a manifestation of a deficiency especially given the decline in these losses in year 2 of the study. Secondly, we observed the delayed form which manifested itself at about 3 weeks of age in otherwise healthy, large, rapidly growing calves. Two maternally-raised calves, one of whom died 21 days postpartum, exhibited substantial daily mass gains initially but began to falter by day 8 (Stephenson et al. 1999); we contend that this was related to the onset of white muscle disease. We successfully treated one these 2 calves with a selenium/vitamin E injection and its condition improved markedly. The second calf died and necropsy confirmed white muscle disease.

Although selenium levels in MRC calves and cows appear normal, vitamin E levels in calves at the MRC fell in the low range observed for cervids in zoos and well below the mean of 2.09 μg/g, (Dierenfeld 1989). Vitamin E levels in adult females at the MRC were orders of magnitude lower than those observed in free-ranging moose on the Tanana Flats during the same time of year. Dierenfeld (1989) recommends that zoo ungulate feeds contain > 200 IU vitamin E/kg total diet to avoid deficiencies. In contrast, prior to 1999 MRC moose feeds contained 5 IU vitamin E/kg.

We hypothesize that the effects of a vitamin E deficiency were manifested during this project because of the high productivity of these animals relative to previous MRC projects. In the past, although female moose at the MRC are routinely bred, they often were not permitted to breed in successive years while maintained primarily on pelleted feeds. High reproductive effort by females increases their vitamin and mineral costs (Robbins 1983). Furthermore, the duration (October through May) that our cows were on only pelleted feed is not typical of past studies with reproductive females at the MRC. In this study pregnant cows did not have access to browse during gestation or the first 2 weeks postpartum;

thus, they were not able to consume lush green vegetation with high vitamin E in spring when supplementation at the end of gestation and beginning of lactation may be critical. Furthermore, animals were observed consuming limited quantities of spruce. The terpenes present in spruce may have increased vitamin E requirements (Dierenfeld 1989). Consequently, in contrast to this study, ungulates with access to abundant natural browse are unlikely to be suspected of vitamin E deficiencies. Furthermore, because vitamin E storage occurs in lipid reserves, seasonal deficiencies are less suspect.

We increased selenium levels in our feeds at the MRC to 0.65–0.7 ppm following identification of a vitamin E deficiency. Because of a sparing interaction that occurs between selenium and vitamin E in the diet (Dierenfeld 1989), higher selenium levels in feed may aid in preventing vitamin E deficiencies as well. Furthermore, vitamin E levels in our moose feeds were boosted to 220 IU/kg.

Predation is routinely identified as the proximate cause of mortality in neonatal moose in Alaska. Factors that contribute to increased vulnerability to predation are rarely considered but may be the ultimate causal factors associated with predation, in some cases (Sinclair and Arcese 1995, Keech et al. 2000). The apparent selenium deficiencies that we observed in cow moose from the Tanana Flats indicate that levels are below recommended levels for livestock and could be a contributing factor related to neonatal losses.

Flueck (1991) illustrated that whole blood selenium levels were representative of glutathione peroxidase activity in black-tailed deer (*Odocoileus hemionus*) erythrocytes. Deficient selenium levels in free-ranging moose were identified in Washington (Hein et al. 1994) and Sweden (Galgan and Frank 1995). Whole blood selenium levels in moose in Washington averaged 0.015 ppm (μg/g; Hein et al. 1994), an order of magnitude below that which we observed in calves at the MRC. Selenium levels in our moose on the Tanana Flats, Alaska, generally fell between the MRC and Washington values but many were deficient. Selenium levels in domestic cattle are considered adequate at >0.1 ppm in whole blood. However, Robbins et al. (1985) hypothesized that wildlife evolved in low selenium environments and may be better adapted to them. In contrast, Flueck (1994) suggested that wild ruminants may be equally susceptible to deficiencies as are domestics. Flueck (1994) documented increases in recruitment in a California black-tailed deer population following supplementation with selenium and established a causal link between selenium deficiencies and depressed reproduction in free-ranging ungulates.

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