MICRONUTRIENT SUPPLEMENTATION IN RELATION TO HAIR LOSS SYNDROME IN CALIFORNIA MULE DEER

By

Max W. Marquez

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Committee Membership

Dr. Richard N. Brown, Committee Chair

Dr. Micaela Szykman Gunther, Committee Member

Dr. Daniel Barton, Committee Member

Dr. Erin Kelly, Program Graduate Coordinator

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ABSTRACT

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The Jawbone deer herd has been monitored on their wintering grounds since the 1950s and recently began decreasing over the last several years. The decline prompted an investigation into the reasons why. Coinciding with the population decline is the appearance of hair loss syndrome and two species of exotic lice. In order to evaluate the effects of hair loss syndrome and the influence of exotic lice infestation, 38 wild does from the herd were used in a modified-before-after-control-impact study. Does were treated with micronutrients selenium and copper via boluses in winter (December 2013-January 2014). Selenium treatment, at a dose of 0.3 mg/day was found to increase the blood selenium level in treatment does by 0.145 ppm. At recapture during spring, does exhibited a decrease in hair that broke under examination during a pluck test to a mean of 13% down from 44% suggesting a decrease in the loss of guard hairs. The control group's hair did not exhibit any significant change in the proportion of hair that broke from the pluck test between initial capture during winter (December 2013-January 2014) to spring 2014. Does that were selenium deficient were found to have a mean louse number 23 times greater than does that were not deficient. No results involving copper, lice or the loss of guard hairs were found to different among groups. This study was

conducted in the aftermath of a catastrophic fire event that may have influenced these findings. These results indicate that selenium deficiency could be an important factor in the effects of hair loss syndrome and exotic louse infestation on the Jawbone deer herd and possibly explaining some of the reason for the herd's decline.

ACKNOWLEDGEMENTS

Thank you to my wife Angie who has supported me throughout this process and for being a wonderful mother to our son Wren while I was away in the field. I would also like to thank the California Department of Fish and Wildlife for funding on this project. Thank you to Greg Gerstenberg for allowing me the opportunity to work on the project. Thank you to my advisor Rick Brown for guidance on the writing process.

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INTRODUCTION

Monitoring of the Jawbone mule deer (*Odocoileus hemionus*) herd, a subset of the Tuolumne deer herd, on their wintering grounds started in the 1950s (Leopold et al. 1951) and has continued through 2020. The herd is migratory; spending summers at high elevations north of, and partially within, Yosemite National Park, Mariposa County, California and they winter in the area of Jawbone Ridge from which they derive their name. The Jawbone ridge is situated between the Clavey and Cherry rivers that flow from Lake Eleanor and Cherry Lake, respectively. The wintering grounds provide important food and cover habitat during winter and spring that ultimately affect herd size. The Jawbone deer herd recently experienced a decline in number stimulating an ongoing study of the potential causes of the decline (G. Gerstenberg, California Department of Fish and Wildlife, personal communication). Importantly, the decline coincided with, and may have been exacerbated by, the recent appearance of hair loss syndrome (HLS) and two species of exotic lice (Roug et al. 2016).

Hair loss syndrome was first described in the late 1990s, appearing on Columbian black-tailed deer (*Odocoileus hemionus columbianus*) throughout much of the coastal mountains of Washington. Hair loss was soon noted in Oregon, then Northern California and eventually on mule deer of the Sierra Nevada Mountains and as far east as the Rocky Mountains (Foreyt 2004, Bender and Hall 2004, Bildfell et al. 2004, Mertins et al. 2011). The first indication that a deer may be experiencing early HLS is the appearance of a rough hair coat. However, the first clear sign is the loss of dark guard hairs that leaves the

lighter undercoat visible; with excessive grooming, HLS can progress to bare skin (Bildfell et al. 2004). Fawns are affected disproportionally by HLS; does experience both lower prevalence and severity of HLS and bucks rarely are affected (Bildfell et al. 2004). Signs of HLS are sometimes accompanied by lethargy, emaciation and even death (Bender and Hall 2004, Bildfell et al. 2004).

Two species of exotic lice, *Bovicola tibialis* and *Damilina spp*., have been associated with HLS (Foreyt 2004, Mertins et al. 2011). More severe infestations of the chewing louse *Bovicola tibialis* have been documented on deer when stressed nutritionally or by winter conditions (Mertins et al. 2011). An animal's ability to resist infections or parasites largely depends on its nutritional health. Furthermore, poor nutrition can negatively affect immunity as well as grooming behavior, which might lead to an increase in the numbers of parasites found on deer (Gomez et al. 2002, Foreyt et al. 2004, Hefnawy and Tórtora-Pérez 2010). Micronutrients are essential components of nutrition and act in many cellular functions of the body. Micronutrients of primary interest for this study were selenium and copper.

Selenium is an essential micronutrient that helps maintain physiological homeostasis by its influence on the immune system (McKenzie et al. 1998, Arthur et al. 2003) as well as by acting as an antioxidant (Turner and Finch 1991, McKenzie et al. 1998, Gomez et al. 2002, Arthur et al. 2003, Hefnawy and Tórtora-Pérez 2010, Flueck et al. 2012). There are many reasons for selenium deficiency in animals, but areas with volcanic soils are inherently deficient in selenium. Volcanic soils are also high in sulfur which competes with selenium during uptake (Flueck 1991, Hefnawy and Tórtora-Pérez

2010, Flueck et al. 2012) potentially exacerbating selenium deficiency. Typically, plants that grow in selenium-poor soils have low levels of selenium in their tissues, and animals that eat these plants may become selenium deficient (Hefnawy and Tórtora-Pérez 2010). Herbivorous mammals are likely to be deficient in selenium when soils contain <0.5 ppm or when vegetation contains <0.0001 ppm (Hefnawy and Tórtora-Pérez 2010).

Livestock are considered severely deficient when their blood selenium is below 0.05 ppm (U. S. Department of Agriculture 2016). In free-ranging mule deer, the low-marginal range for blood selenium was reported to be 0.07 ppm; below this is when deficiency is believed to begin to occur (Flueck 1994), and this level of deficiency was previously associated with HLS in the Jawbone deer herd (G. Gerstenberg unpublished data).

Selenium deficiency, similar to HLS, disproportionally affects fawns and does (Flueck 1994) because selenium is imparted from doe to fawn during gestation and the preweaning period, which may further reduce selenium blood levels in the doe. One study, which investigated selenium's influence in deer, found that does treated with selenium were three times more likely to be accompanied by a fawn the following year (Flueck 1994) illustrating the importance of selenium on deer reproduction. However, due to the complexity of micronutrient interactions, selenium's importance in homeostasis is not fully understood.

Selenium functions as a modulator of the immune system where it is linked to its effects on neutrophils and lymphocytes (Arthur et al. 2003). Interestingly, the neutrophils of selenium-deficient animals have diminished ability to kill phagocytized pathogens;

neutrophils of selenium-deficient animals phagocytize pathogens as well as those of animals with sufficient levels of the micronutrient but fail to kill the pathogens once phagocytized (Boyne and Arthur 1986). This difference results from a decrease in cytosolic glutathione peroxidase activity (GPx) that facilitates antioxidant processes, which clears cells of free radicals produced in the oxidative burst (Boyne and Arthur 1986, Flueck 1991, McKenzie et al. 1998, Arthur et al. 2003). The GPx process allows neutrophils to continue the oxidative burst for an extended time without causing self-harm. GPx activity of neutrophils is dependent on the amount of selenium present in the body (Arthur et al. 2003). Oxides build up within the cells causing oxidative stress and decreased function when intracellular selenium is unavailable. Selenium also affects the ability of both B and T lymphocytes, to undergo clonal expansion as part of the acquired immune response (Turner and Finch 1991, Arthur et al. 2003), and the supplementation of selenium to deficient animals can restore normal function.

The primary literature offers little information on the effects of copper deficiency in wild ruminants and almost nothing concerning copper deficiency in mule deer. However, the effects of copper, much like selenium, have been well understood in domestic livestock for some time, and young livestock are known to suffer more severely than adults (McDowell 1985). Copper deficiency is typically associated with failure to gain weight, anemia, diarrhea, lower birth rates and change of hair pigmentation. When copper deficiency affects coat color, the coat appears lighter due to reduced pigmentation (Stelter 1980, Miller et al. 2001, Humann-Ziehank et al. 2008, Handeland et al. 2008). Copper deficiency can be either primary or secondary. Primary deficiency occurs when

adequate amounts of a particular nutrient are not available in the environment (i.e., browse) leading to low intake and subsequently low levels of copper in the liver or blood. Secondary deficiency occurs when nutrient levels are adequate in the environment, but there are competing minerals that inhibit the absorption and bioavailability of the desired nutrient. Minerals that compete with copper include iron, sulfur, and molybdenum (Handeland et al. 2008). Similarly, these same minerals can also inhibit the absorption of selenium. The acceptable amount of copper in the blood of red deer (*Cervus elaphus*) has been estimated to 0.3-1.5 ppm; when levels fall below the low end of this range it is possible to see the effects of deficiency (Handeland et al. 2008).

Another micronutrient of interest is zinc. Like selenium, when deficient in zinc the immune system becomes depressed and the individual becomes more susceptible to infection (Shankar and Prasad 1998, Bartoskewitz et al. 2007). Zinc contributes to immunological response of the skin as well as cellular non-specific immunity (Shankar and Prasad 1998). Without adequate levels of zinc in the blood the immunoglobulin response may be delayed allowing for infections to spread (Salvin et al. 1987).

Micronutrient deficiencies can influence fecundity as well as fetal and fawn survival in ungulates (Flueck 1994, Miller et al. 2001, Handeland et al. 2008), and malnutrition may be exacerbated by harsh weather patterns, infectious diseases, and perhaps HLS. The combination of micronutrient deficiencies and HLS could be affecting reproduction and fawn recruitment in mule deer, and therefore partly account for the decreasing size of the Jawbone deer herd.

The focus of this study was to investigate whether supplementation with micronutrients would reduce louse infestations and possibly slow the progression of HLS in California mule deer. To do this, I aimed to associate the loss of guard hairs with micronutrient concentrations in the blood. Additionally, I explored micronutrient associations with the presence and numbers of exotic lice reported to cause HLS and possible impacts of micronutrients on recruitment in the herd. I tested the following predictions between micronutrients, hair loss, and lice.

(1) Selenium in whole blood at the time of capture would be negatively associated with the loss of guard hairs and the onset of HLS; does with adequate selenium levels, would be less likely to show signs of HLS than does in the deficient range.

(2) Because exotic lice have been shown to cause HSL elsewhere, I predicted I would find a positive correlation between the abundance of lice and the development of HLS.

(3) Because selenium can be important for adequate immune responses, I predicted that blood levels of selenium would have a negative relationship with louse abundance.

(4) I predicted that does treated with micronutrients during the preceding winter would return to the wintering grounds with more fawns per doe than would does from the general untreated population because adequate levels of selenium and copper are necessary for successful reproduction and early fawn growth.

STUDY LOCATION

This study was located on Jawbone Ridge (10S 0759557E 4196132N), which lies just to the west of Yosemite National Park in the Sierra Nevada Mountain Range of Tuolumne County, California. The wintering grounds of the Jawbone Ridge deer herd is referred to as the having two sides: the "Clavey" side and the "Cherry" side, in reference to the main rivers that flank the ridge and define its shape (Figure 1) (Leopold 1951).

The Jawbone Ridge is comprised of a network of open meadows that spread out over the wintering grounds with some surrounded by trees while others defined by brush. The meadows are home to many wildflowers including pansy monkey flower (*Mimulus pulchellus*) and white monkey flower (*Mimulus bicolor*). The introduced bulbous blue grass (*Poa bulbosa*) as well as other short grasses grow in and around the meadows. The most common brush types are buck brush (*Ceanothus ceneatus)*, deer brush (*Ceanothus integerrimus*), western mountain mahogany (*Cercocarpus betuloides*), and manzanita spp. (*Arctostaphylos spp.*). Common trees include ponderosa pine (*Pinus ponderosa)*, Jeffrey pine (*Pinus jeffreyi)*, sugar pine (*Pinus lambertiana)*, gray pine (*Pinus sabiniana)*, blue oak (*Quercus douglasii)* and black oak (*Quercus kelloggii)* (Leopold 1951).

The Rim Fire burned greater than 104,000 ha, including 90-95% of the Jawbone herd's wintering range during late summer 2013 (Sierra Nevada Conservancy 2018), leaving dramatic change in its wake. Deer that migrated westward from their summer range in the high country that fall faced a heavily damaged and fragmented habitat.

However some fire skip did occur, leaving portions of intact forest. The largest area spared by the fire is known as the Lava Cap; a large patch of meadow and mixed pine-oak forest (~ 1.5 km^2) near the top of Jawbone Ridge. By necessity, this study focused on the deer using remaining habitat, including the area of the Lava Cap.

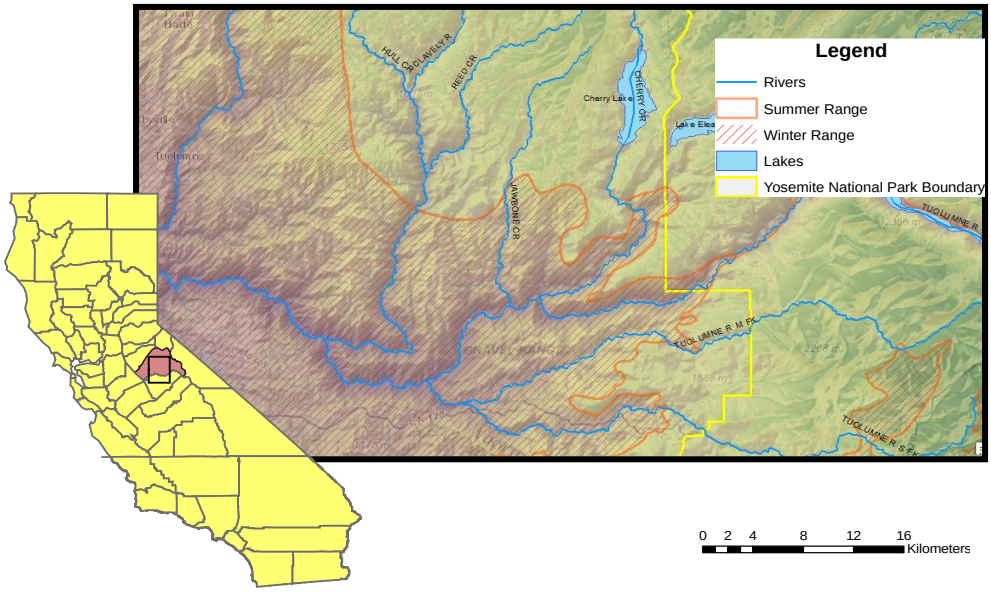


Figure 1: Map of the Jawbone study area in Tuolumne County, CA, modified from Berger (2015). Deer winter along the ridge between the Cherry and Clavey rivers and spend summers north and east of the orange line.

Large portions of the Jawbone wintering grounds occur on volcanic soils, as well as in Tuolumne County. Selenium soil concentration ranges between 0.15 to 0.17 ppm for Tuolumne County (Figure 2; U.S. Geological Survey 2017).

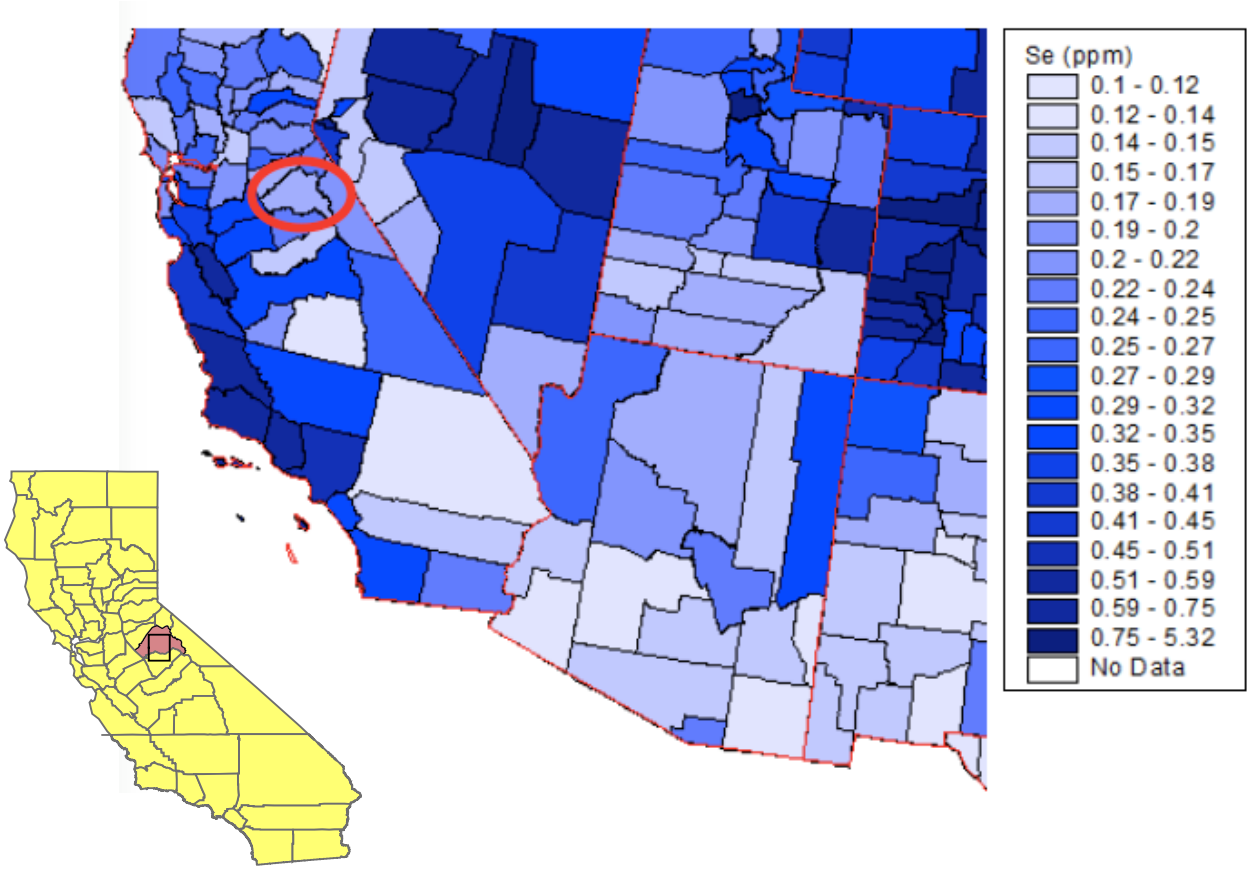


Figure 2: Map of the selenium concentration of Tuolumne County in relation to counties of the Southwest United States. Modified from the USGS home page (USGS 2017).

METHODS

Capture and Sampling

A modified-Before-After-Control-Impact (BACI) sampling design was used for this study. Animal capturing and handling occurred in two occasions: the first in December 2013 to January 2014 (Winter) and the second in March-April 2014 (Spring), each lasting approximately two weeks.

Clover traps were positioned, disguised and baited with a mix of alfalfa and mistletoe (Thompson et al. 1989, Schemnitz et al. 2009). Traps were set with a modified triangular trip wire as a trigger. This style of trip wire was easily adjusted for tension and placement within the trap. All clover traps were checked twice a day, once in the morning and once in the evening. During capture, traps were approached as quickly as possible and collapsed in a controlled manner to avoid injury of the deer. The trapped deer was physically restrained at which point eye covers were placed over the muzzle if possible or laid over the face. Chemical immobilization was administered by intravenous injection of xylazine (2.2-4.4 mg/kg). Once sedated hobble restraints were applied and the deer was removed from the trap. Free range darting (Pneu-Dart, Williamsport, PA) was also used to supplement captures of target animals. Experienced professionals from California Department of Fish and Wildlife, led by Greg Gerstenberg, conducted all darting. Deer were darted with a combination of drugs containing Telazol (2-3 mg/kg) and xylazine (2-3 mg/kg). Body weight was estimated to allow for proper dosage. Sedated deer were

approached cautiously and quietly then physically restrained. Eye covers and hobble restraints were applied to minimize stress on the deer and for researcher safety.

Captured animals were processed for measurements and samples. Vital signs, including body temperatures, heart rates and respiration rates were monitored while the animals were sedated. Immobilized deer were weighed using a Poluze livestock spring scale (Rubbermaid, Saratoga Springs, NY). Neck circumference was measured at three locations: behind the jaw, mid neck and base of the neck. Total body length from nose to base of the tail was measured as well as girth, taken directly posterior to the elbow. I assessed body condition based on a scorecard system developed by CDFW (Appendix A). Hair samples were collected by plucking and examined for signs of brittleness or other abnormalities. A pluck consisted of at least 10 hairs. To assess brittleness thought to be related to hair loss, hair was plucked from 5 regions of the body: the rump, back, shoulder, neck and chest. Each hair in the pluck was examined to determine if it was removed from the follicle, broke at the follicle or broke midway along the shaft. Hair coat was also examined for preclinical signs of hair loss syndrome that manifest as hair looking scruffy, wet, or dull.

Whole blood samples ($\leq$ 20 cc) were drawn by jugular venipuncture and blood was divided into tubes containing either the anticoagulant ethylene-diamine-tetraacetic acid (EDTA) or lacking anticoagulant. Blood in tubes without EDTA was allowed to clot before the serum was separated by centrifuge for 10 minutes at 3,000 rpm. Serum vials and tubes containing EDTA were stored at -18 C until sent to the California Animal

Health and Food Safety Laboratory System at the University California Davis for analysis.

GPS receivers (Advanced Telemetry Systems, model 2110 with a mean life of 586 days and mortality signals with an associated VHF transmitter) were secured to all does captured during winter trapping: GPS data will be published elsewhere and were not used for this study. Collars were fitted with room of the width of a hand laid on the neck of the deer. The extra room was to allow for weight fluctuations, as the deer were thin at capture, likely due to the effects of the Rim Fire. Transmitter functional status was checked with a receiver to ensure that it was in working order before it was attached to a deer.

Upon completion of data collection, tolazoline (4.4 mg/kg) was administered intravenously to reverse the effects of the xylazine. The dosage of xylazine used for chemical restraining must be allowed to dissipate in the animal's system for no less than 40 minutes following immobilization before the addition of tolazoline (Monteith et al. 2012).

Hair Loss Score

All deer caught were assessed for extent of hair loss and given a score, on a scale from 0-5, using the Central Region Hair Loss Method (Berger 2015) developed by Greg Gerstenberg (Appendix B). The scoring method depicts the overall hair loss on the animal by how characteristics of each body region affected by hair loss combine. There were five regions evaluated in this study: head and neck, back, sides, belly and groin, and

the rump. All five regions were examined on every target deer while in hand. Hair loss scores were assessed during capture as well as between capture occasions using spotting scopes and binoculars. To be included in the data, I needed to be able to visualize the entire side of the deer; if this was not possible then I would record hair loss as + or -. In order to check the validity of hair loss scores at distance, they were compared to scores while in hand. When possible, deer were given two hair loss scores, one from a distance taken by spotting scope or binoculars immediately preceding capture and one while in hand. To document the progression of hair loss, visual assessment on each study animal was attempted at least once in February and twice in both March and April 2014.

Ectoparasite Evaluation

A 10 cm^2 sampling square was used to standardize inspection for lice. The square was placed randomly within each of the five regions of the body noted above. All lice were removed with a louse comb and retained in ethanol for identification and enumeration. If no lice were found in the five sampled areas, then the animal was further examined to establish louse presence or absence. Louse specimens were shipped to the National Veterinary Services Laboratories in Ames, Iowa, for identification.

Micronutrient Supplementation

Each doe captured during the December 2013-January 2014 (Winter) capture occasion was treated with two micronutrient boluses. The first was a selenium bolus (Pacific Trace Minerals, Sacramento, CA; SE365 boluses release 3 mg Se/day for 12

months). The second was a copper bolus (Copasure, Animax, Stanton, England; copper boluses were adjusted to provide 0.4 g Cu/kg body weight). Deer were monitored as they revived from sedation, and, once the swallow-reflex had returned sufficiently, a balling gun was used to deliver the boluses into the upper esophagus.

Radio Tracking

Monitoring of deer by VHF was conducted from January 2013 to May 2014, after which the deer began to migrate to higher elevations. Monitoring activities included a daily mortality check and a weekly triangulation of an animal's location and biweekly visual assessments of the progression of HLS. Mortality signals were broadcast if the deer remained inactive for more than 8 hours. For telemetry, a Communication Specialist (R-1000, Orange, CA) receiver and two types of antennas, directional (Telonics, RA-23K, Mesa, AZ) and omnidirectional (Telonics, RA-5A, Mesa, AZ), were used.

Reproduction

Reproductive data were collected the following winter 2014-2015 after the does returned from their summer grounds. Fawns were considered associated with does when no other deer was visible or when a fawn remained in close proximity and mirrored the doe's movements. I drove all accessible roads of the study area daily for one week (January 2015) and recorded all fawns and does observed that met the criteria mentioned above. Counts were only recorded once for a given area to avoid double counting the unmarked general population. The term "area" is used loosely here, meaning that same

stream, draw or hillside within a distance approximately equivalent to that of deer's home range. If a doe and fawn were seen more than once in the same portion of the study area then it was recorded and it was assumed for this study that area was part of the fawn and doe's territory. The highest number of fawns was recorded for each area.

Composition Counts

Composition counts have been conducted biannually on Jawbone Ridge during the past 50 years to estimate the relative abundance of deer on the wintering grounds. The counts are done in early December when most deer have migrated to the lower elevation winter range, and again in April before the return trip to the summer grounds has begun. Volunteers and field personnel are enlisted to conduct the composition counts. Counts have followed strict procedural methods throughout the winter range, with emphasis on the Lava Cap due to high numbers of deer and visibility. Nineteen different routes encompassing much of the winter range have been monitored during composition count surveys. Survey routes traversed the road system throughout the herd's winter grounds and each route covered a unique portion. Routes were generally surveyed simultaneously on a single day to minimize double counting. The majority of composition count surveys have been conducted by vehicle except around the Lava Cap, which has been surveyed on foot due to easy access.

STATISTICAL ANALYASIS

To examine the effects of micronutrient treatment on mule deer does, I constructed before and after test groups using does caught during both capture periods. For the control group, I used 31 does from the winter capture period (before) and the nine newly captured does from the spring capture period (after). For the treatment group, I used eight does that were caught in the initial winter capture period (before) and then re-caught again in the spring (after), creating a quasi-paired sample. All 39 deer caught during the initial winter capture received both selenium and copper bolus treatments. Micronutrient levels for control does were sampled before treatment. The does that served as the 'after' sample for the control group had not received treatment.

A logistic regression with a Bernoulli distribution was used to check associations with selenium, copper, and zinc among the spring captured does to test the influence of micronutrients on the presence of alopecia (absence or loss of hair) (Dai et al. 2013). The same statistical test was also used when examining alopecia and the percent of hair that broke during the pluck tests. A Chi-squared test was used to check the models goodness of fit. I used a two-way ANOVA to look at interactions between test groups for hair breakage. Logit transformation has been reported as a better way to analyze categorical or percent data (Dixon 2008, Jaeger 2008). Because of this, I analyzed the data first using the untransformed value of percent of hair that broke; then I used the logit transformation. I checked model assumptions with the Shapiro-Wilks normality test and Bartlett's test for homogeneity of variance (Noughabi 2016). I report here on the results

for the raw data of percent hair that broke as it conformed to the model assumptions where the logit model did not.

Six linear models were constructed to examine the effects of the micronutrients selenium, copper and zinc on the percent of hair that broke during the examinations. The percent of hair breakage was logit-transformed to conform to the normality assumption. The six models were evaluated in a stepwise manner and ranked using AICc scores; AICc was used because of small sample size.

A negative binomial regression was conducted between the number of lice and ppm blood concentration level of each micronutrient of interest and a categorical variable deficient (does with selenium 0.7 or less). Initially, a zero-inflated negative binomial model was considered. However, the negative binomial model was chosen because the data failed to converge with the zero-inflated model. The R packages ggplot2, pscl, MASS and boot were used to conduct the analysis.

Linear regression was used to evaluate the relationships of the blood levels of selenium, copper and zinc to the number of biting lice found on the deer. I tested normality assumptions with a Box-Cox test and log transformed the data to meet normality. One outlier doe was removed from the analysis as Cook's distance revealed it had an inordinate amount of influence on the model. A generalized linear model with a Poisson distribution was considered for analysis on louse intensity, but it exhibited over-dispersion, so it was not used in favor of a simple regression.

T-tests were used to examine the blood levels of selenium, copper, and zinc in both treatment and control groups, as well as in relation to changes in weight of does with

and without the presence of alopecia, the presence of lice, and between does whose coat was normal or dull.

I conducted a Mann-Whitney-U test to compare reproduction between the treated does and does from the general population in spring 2015 based on the number of fawns that returned with does from the summer grounds.

I evaluated the potential effect of the Rim fire on herd size by comparing the mean number of deer from spring 2013 (before the fire) and spring 2014 (after the fire) composition counts (unpublished data, G. Gerstenberg, California Department of Fish and Wildlife).

RESULTS

A total of 57 target animals were caught during 2 capture periods: 39 does were captured during the winter capture period and 18 were captured during the spring capture period. All 39 does from the winter capture period were treated with micronutrients, but one regurgitated its selenium bolus, leaving a total of 38 does that received micronutrient supplementation during winter (December 2013 to January 2014) captures. The spring capture effort resulted in 9 does that were caught for the first time and the re-capture of 9 does from the initial winter capture. One of the recaptured does was released early due to heat-stress, and its hair was not assessed for brittleness, leaving only 8 of the recaptured animals fully processed (Table 1).

Table 1: Mean blood levels of selenium, copper and zinc, body weight, body condition, body length, louse prevalence, louse intensity and percent of hair that broke from the pluck test involving California mule deer captured in December, 2013,and January, 2014, (winter) and March through April of 2014 (spring) on the Jawbone Ridge winter range in Tuolumne County, California.

	Capture period			
	Winter		Spring	
	Control[a] n=31	Treatment n=8	Control[b] n=9	Treatment n=8
Selenium (ppm)	0.10	0.10	0.09	0.25*†
Copper (ppm)	1.47	1.52	0.93	0.97
Zinc (ppm)	0.99	1.00	1.31	1.34

	Capture period			
	Winter		Spring	
	Control[a] n=31	Treatment n=8	Control[b] n=9	Treatment n=8
Weight (kg)	50.80	48.08	51.42	50.51
Body condition	2.15	1.75	1.88	1.80
Body length (cm)	141.40	137.50	139.00	137.50
Louse intensity	15.00	2.00	40.00	159.00*†
Louse prevalence (%)	19	11	78*†	44
Hair broke (%)	40	44	25	13†

[a] Animals in this group were sampled prior to treatment, treated with micronutrient boluses and never recaptured.
[b] Animals in this group were captured for the first time during spring, 2014.
* Denotes a significant difference between groups (Control vs Treatment).
† Denotes a significant difference within groups (Control vs Control; Treatment vs Treatment).

Alopecia

Four re-captured does and 4 newly captured does caught during spring 2014 showed signs of alopecia. There was little to no evidence that blood concentrations of selenium ($z = -1.46$, $p = 0.15$), copper ($z = 0.30$, $p = 0.77$) or zinc ($z = 0.39$, $p = 0.39$) influenced the presence of alopecia. Likewise, percent of hair that broke from the pluck test was not found to predict alopecia ($z = 1.39$, $p = 0.16$).

Visual sightings from 27 of the 38 previously treated does during February, March and April resulted in an assessment that 2 does showed mild signs of alopecia;

both were rated at a severity level of 1 out of a possible 5. Both of these does were re-captured during the spring; however, once in hand, one of their scores was increased to 3 and one increased to 5 (Table 2).

Table 2: Comparison of paired alopecia scores from 19 California mule deer does (designated by letters A through P) captured in Spring 2014 with previous visual scores obtained from a distance using a spotting scope within the 2-week capture period. Visuals and captures were conducted before the migration, in mid-May, from the Jawbone Ridge winter range in Tuolumne County, California.

	Control										Treatment								
Alopecia score of each doe	A	B	C	D	E	F	G	H	I	J	H	I	J	K	L	M	N	O	P
At distance	NA†	0	0	0	0	0	0	0	0	0	NA†	0	0	0	0	1	1	0	0
In hand	0	0	3	5	1	5	0	5	0	0	1	0	0	0	0	3	5	0	0

† Not accessed

Hair Loss

Tests of hair brittleness, conducted during the winter captures, resulted in hair that broke at a mean of 44% across all regions of the body sampled. Hair that broke exhibited a significant decrease to a mean of 13% during spring for treated animals ($F_{3, 52} = 4.25$, $p = 0.006$). The control group's hair did not exhibit any significant change in the mean proportion of broken hairs across all regions of the body between the initial winter

capture period (40%), and spring recapture (25%) ($F_{3, 52} = 1.31$, $p = 0.11$). There was no difference in the percent of hair from treatment and control does that broke during spring ($F_{3, 52} = 4.252$, $p = 0.24$) (Figure 3).

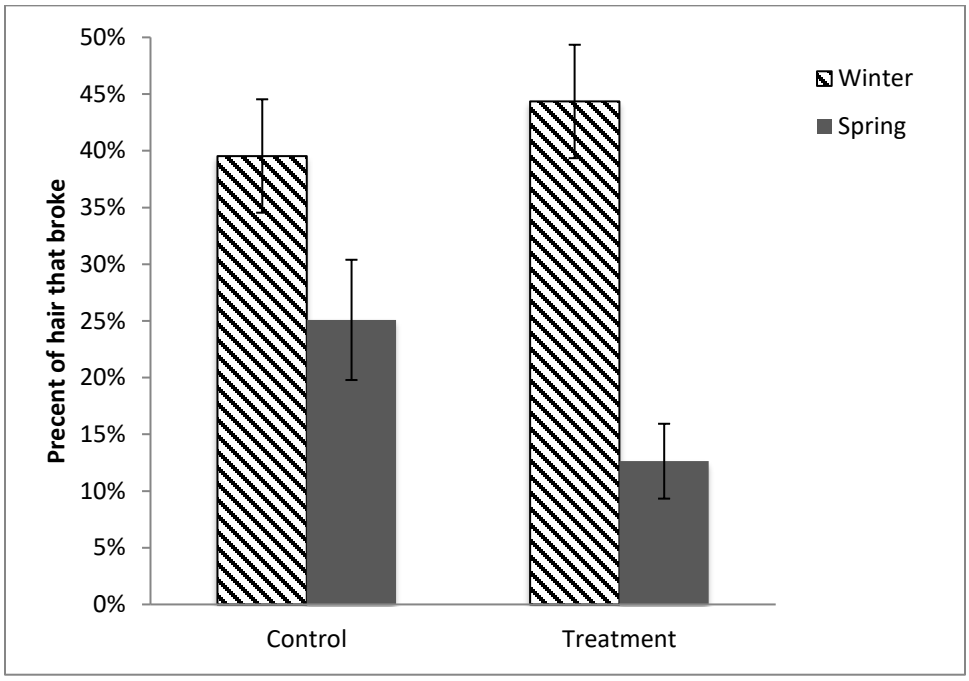


Figure 3: Mean value of percent hair that broke from breakage tests across all regions of the body. Data from all body regions were pooled to acquire the total number of hairs (n) removed during the test. Data collected from both winter (December, 2013, and January, 2014) and spring (March-April 2014) capture periods on the Jawbone Ridge winter range in Tuolumne County, California.

Using model selection criteria (Akaike's Information Criterion adjusted for small sample size, AICc), on a regression analysis, the simplest model involving only selenium accounted for 80% of the weight as well as the lowest AICc score (Table 3). However, selenium was found to have little influence on hair breakage; for each 1-unit increase in blood selenium concentration, the percent of hair that broke decreased by only 0.022% (95% CI: 0.007 to 6.234).

Table 3: Model selection to assess the influence of micronutrients on hair brittleness, determined by a hair breakage test, from all Spring-caught California mule deer does. Spring capture consisted of both treatment and control animals. Data collected from both winter (December, 2013, and January, 2014) and spring (March-April 2014) capture periods on the Jawbone Ridge winter range in Tuolumne County, California.

Model	*K*	AICc	△AICc	Weight
Percent of Hair Breakage				
Selenium	2	87.1	0	0.802
Selenium + Copper	3	90.2	3.1	0.170
Selenium + Copper + Zinc	4	94.2	7.1	0.023
Selenium * Copper + Zinc	5	98.7	11.6	0.002
Selenium + Copper * Zinc	5	99	11.9	0.002
Selenium * Copper * Zinc	7	117.8	30.7	0.000

Lice

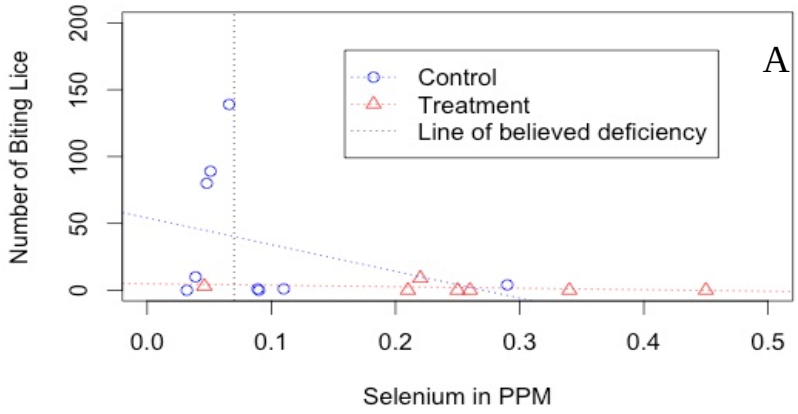
The prevalence of biting lice increased between the winter capture period to the spring capture period; control does exhibited a change in prevalence from 19% in winter (n =31) to 78% (n = 9) in spring whereas treatment does exhibited a change from 11% (n = 8) in winter to only 44% (n = 8). The intensity of louse infestation showed no significant change in control does with a mean of 15 lice observed during winter examinations to a mean of 40 lice observed during spring examinations. The mean intensity of infestation showed an increase in treatment does rising from 2 in winter to 159 in the spring (Table 4). Results of the regression model using intensity of infestation of does with lice present indicated no clear influence by the micronutrients selenium, copper or zinc on the numbers of lice.

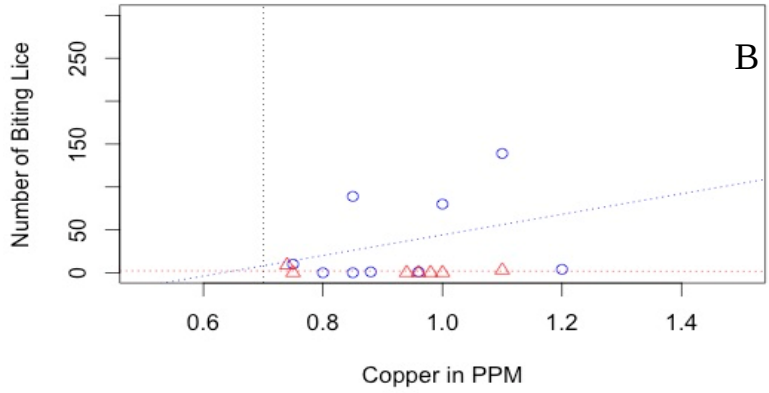
Table 4: Prevalence and mean intensity of lice from California mule deer does. The Treatment group was treated with boluses of selenium and copper during the winter capture season. Data collected from both winter (December, 2013, and January, 2014) and spring (March-April 2014) capture periods on the Jawbone Ridge winter range in Tuolumne County, California.

Group	Prevalence of deer with lice	Mean Intensity of lice on infested does
Winter Control n = 31	19%	15 (0 - 20)
Spring Control n = 9	78%	40 (0 - 139)
Winter Treatment n = 8	11%	2 (0 - 2)
Spring Treatment n = 8	44%	159* (0 – 619)

*Mean number driven by lice on a single individual. Without this individual, the mean for the group is 2

The maximum number of lice removed from a single doe in the spring-caught control group was 139, while the maximum number of lice removed from the recaptured treatment does was 619. However, as mentioned above, this latter individual was an extreme outlier and was removed from this calculation. Louse maxima from winter captured does were 22 and 8 for treatment and control, respectively. Spring caught does had median louse numbers of 0 for treatment recaptures and 4 for control does. Micronutrients did not explain any of the variation in the numbers of biting lice (selenium: $r = -0.05$, $p = 0.25$; copper: $r = -0.07$, $p = 0.33$; zinc: $r = 0.12$, $p = 0.26$) (Figure 4).


A
Control
Treatment
Line of believed deficiency
Number of Biting Lice
0
50
100
150
200
0.0
0.1
0.2
0.3
0.4
0.5
Selenium in PPM


B
Number of Biting Lice
0
50
150
250
0.6
0.8
1.0
1.2
1.4
Copper in PPM

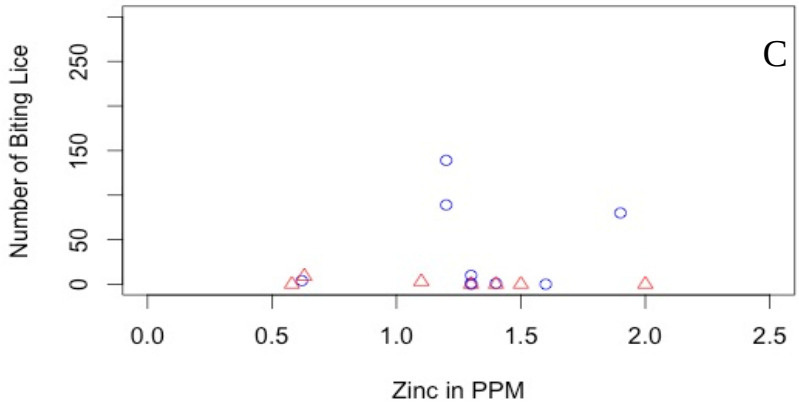

C
Number of Biting Lice
0
50
150
250
0.0
0.5
1.0
1.5
2.0
2.5
Zinc in PPM

Figure 4: Blood micronutrient values for selenium (A), copper (B), and zinc (C) in relation to the number of biting lice removed from Spring-caught California mule deer does. All spring (2014) caught does were used in the sample. The selenium and copper plots have been demarcated with the presumed point of deficiency used in this study. Data collected on the Jawbone Ridge winter grounds, Tuolumne County, California.

More lice, both sucking and biting, were removed from selenium deficient does than from does with a blood selenium concentration greater than 0.07 ppm ($z = 3.17$, $p = 0.01$); 23 times (95% CI: 2.1 to 271 times). There was weak evidence that zinc may influence total louse numbers ($z = -1.79$, $p = 0.07$). Does with higher amounts of zinc in their blood were found to have somewhat fewer lice; with each 1-unit increase in zinc ppm total lice decreased by 16% (95% CI: 0.02 to 1.21 times)

Three of the 9 treated does had sucking lice when caught in spring; 2 of the does had only a single louse and the third had 20 sucking lice found from all areas of her body sampled. No sucking lice were found on any of the does captured for the first time during spring.

Supplementation of Deer with Micronutrients

At initial winter capture, prior to administering of selenium boluses, does in the treatment group had mean blood selenium value of 0.10 ppm (range 0.03-0.14). The same treatment does had mean blood selenium of 0.25 ppm (range 0.07-0.45) at spring recapture, a concentration increase of nearly 2.5 times ($t = 3.38$, $p = 0.004$) (Figure 5). Control group does caught during winter capture had mean blood selenium concentration of 0.10 ppm (range 0.04-0.17) similar to the mean concentrations of 0.09 ppm (range

0.03-0.11) found in the spring, showing no significant difference from the levels recorded at initial capture.

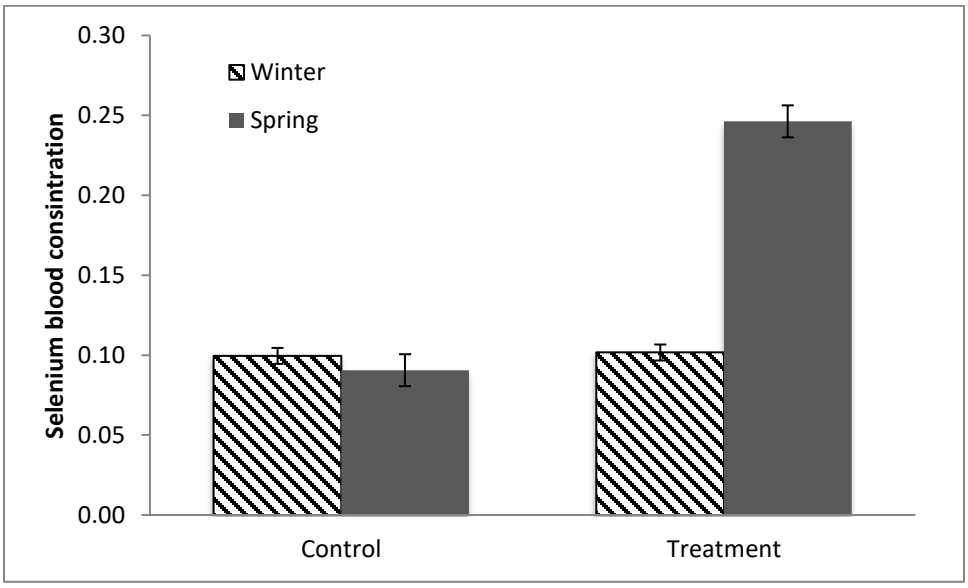


Figure 5: Mean blood selenium levels in ppm of California mule deer. Data collected from both winter (December, 2013, and January, 2014) and spring (March-April 2014) capture periods on the Jawbone Ridge winter range in Tuolumne County, California

Blood copper concentrations of treatment does in winter had a mean of 1.52 (range 0.61-2.0) (Figure 6). In spring, treatment does had mean blood-copper level of 0.97 (range 0.74-1.4); blood copper concentrations were lower in the spring than in winter ($t = -2.6$, $p = 0.015$). At initial capture in winter, control does had mean blood-copper concentration of 1.47 (range 0.06-2.5). In spring, the general population of does that served as a control had a mean blood-copper concentration of 0.93 ppm (range 0.75-1.2). Again, there was a decrease in blood-copper from winter to spring within the control group ($t = -4.95$ $p = 0.008$). However, there was no difference between blood-copper levels of treatment and control does in the spring.

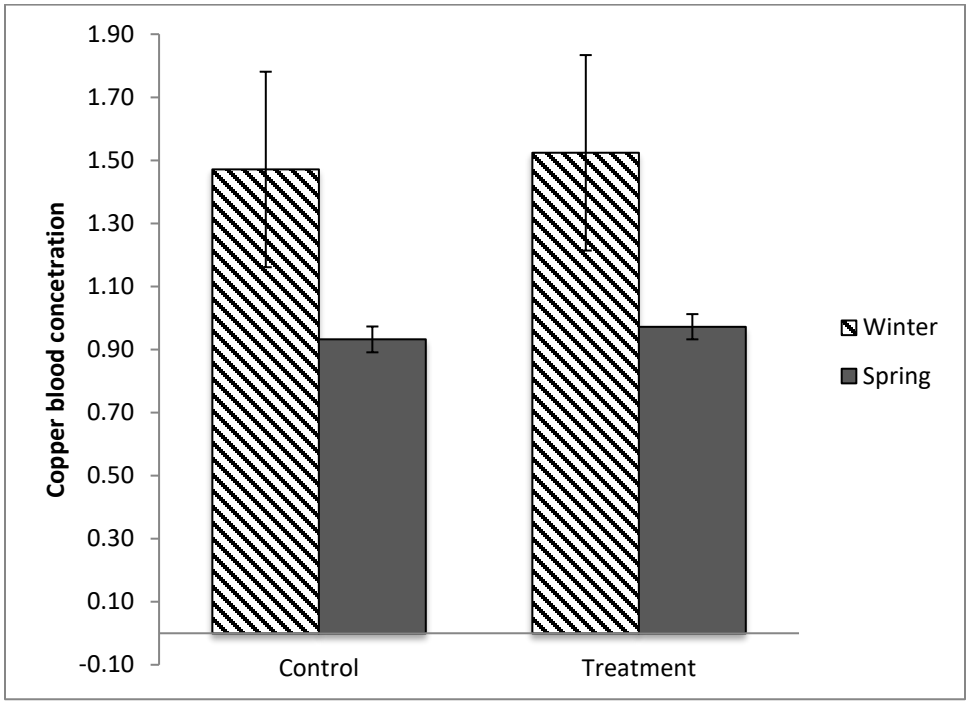


Figure 6: Mean +/- SE blood level of copper in ppm of California mule deer does. Data collected from both winter (December, 2013, and January, 2014) and spring (March-April 2014) capture periods on the Jawbone Ridge winter range in Tuolumne County, California.

Both treatment and control groups, at initial capture, showed similar amounts of zinc in the blood (Figure 7). Zinc blood levels increased in parallel (but non-significantly) for both treatment and control when checked again in the spring, suggesting that treatment of selenium and copper did little to influence the mean blood-levels of zinc.

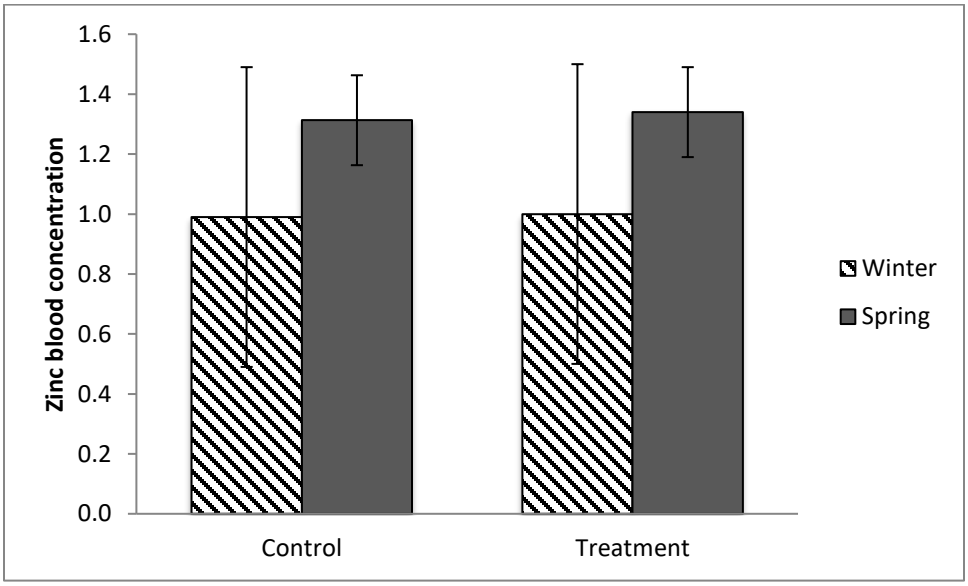


Figure 7: Mean blood zinc levels in ppm of California mule deer does. Data collected from both winter (December, 2013, and January, 2014) and spring (March-April 2014) capture periods on the Jawbone Ridge winter range in Tuolumne County, California.

Reproduction

I observed 34 does during January 2015 that could be assigned a status of either having or not having a fawn. Observations included 13 does treated previously in 2014 and 21 does that were not previously captured. Seven of the 13 (61%) treated does were accompanied by a fawn the following winter, compared to 11 of the 21 (52%) new does that were accompanied by a fawn. I observed one instance of twins in the treatment does and 4 instances of twins in the new does. There was no evidence to support a difference between groups ($z = -0.05$, $p = 0.96$). Likewise, the fawn to doe ratio was very similar between the treated (0.69 fawn/doe) and untreated does (0.71 fawn/doe)

Composition counts conducted during spring, 2013 resulted in estimates of 355 deer: 270 adults and 85 fawns. Similar counts during the post-fire spring of 2014 resulted in an estimated herd composition of 216 deer; 189 adults and 27 fawns (Figure 8), a 39% decrease from 2013 ($t = 2.10$, $p = 0.13$)

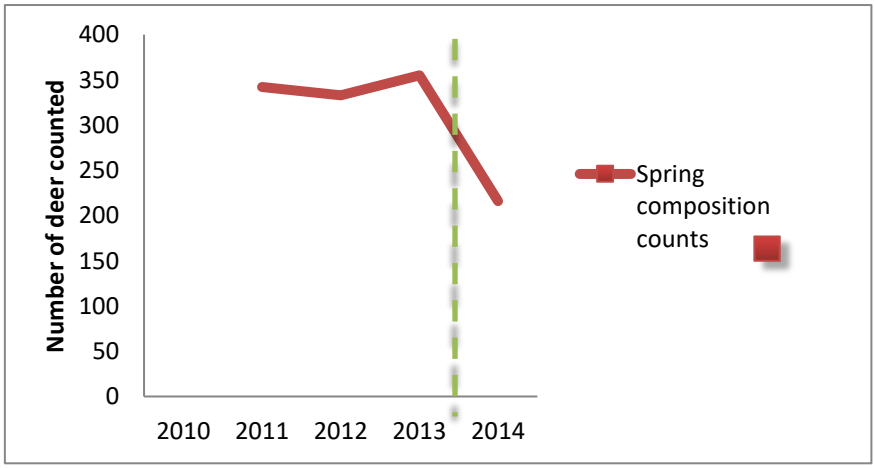


Figure 8: Total number of deer counted during the spring composition counts for years 2010 until 2014, conducted on the Jawbone Ridge portion of the Tuolumne deer herd winter grounds. The dotted line indicates the approximate date of the Rim Fire.

Body Condition

The mean body condition scores from the winter captured treatment and control group does were 1.75 and 2.15, respectively. Body condition scores decreased slightly, but not significantly among control does in the spring to a mean 1.88. The mean body weights of the treatment and control animals, from winter capture, were 48.1 kg (sd = 3.15) and 50.8 kg (sd = 5.55) respectively. Deer caught in spring had mean body weights of 51.4 kg (sd = 2.73) and 50.5 kg (sd = 6.21) for treatment and control groups, respectively. The three variables concerning the presence of lice, alopecia and dull coat were not significantly associated with the weight of does (df =17, p = 0.22).

DISCUSSION

Supplementation with selenium boluses had the intended effect of raising the blood selenium concentration of treated deer; the mean concentration of selenium in the blood increased from 0.10 to 0.25 ppm, well above the 0.07 ppm threshold I considered as deficient. The mean blood selenium concentration of untreated deer caught in April was still low-adequate at 0.09 ppm. After supplementation with selenium, concentrations of the micronutrient in the blood of treatment does were similar to levels reported in white tailed deer in Michigan (Ullrey et al. 1981). A study conducted in Shasta County,

California, across three years on black tailed deer, found the mean blood selenium level to be 0.04 $\pm$ 0.03 ppm (range 0.009-0.119) (Flueck 1991). While for domestic sheep (*Ovis aries*) the accepted deficiency value of 0.05 ppm may hold true (Pugh and Baird 2012, U.S Department of Agriculture 2016), black-tailed deer are a different species and their threshold for deficiency-related disease may differ from sheep. GPx activity was shown to have a linear (r^2 =0.91) relationship with blood selenium, where activity is near zero around 0.03 ppm blood selenium (Flueck 1991). It is possible that the blood selenium level of 0.07 ppm is still within the normal range for mule deer. Levels may have to drop further than what I considered deficient to see any deleterious effects.

An interesting result was the difference in hair breakage among groups. The treatment group showed a significant decrease of breakage from 44% in winter down to 13% in April. The 0.0002% decrease in the propensity of hair to break with an increase of 1 ppm blood selenium is a small but non-zero relationship. This suggests that selenium may play a role in the breaking of hair associated with HLS. A larger sample may be needed to fully explore the interaction of HLS and selenium.

There was a significant decrease in the propensity of hair to break after treatment with micronutrients. The finding of decreased hair breakage in the spring is confounded with concurrent treatments of copper and selenium, which didn't allow me to evaluate the different effects of the two micronutrients. Additionally, this experiment was conducted on a wild population with deer that varied in nutritional status both before and after a large fire that dramatically changed the available forage. Nonetheless, treatment with

selenium raised blood selenium values above the presumed threshold of deficiency and fewer hairs broke after than before treatment.

I found no direct link between selenium and alopecia. The negative effects of selenium deficiency has been documented in livestock, lab animals, as well as free range animals in the form of either white muscle disease, decreased survival of young, or poor wool production (Seppard and Grant 1984, Turner and Finch 1991, McKenzie et al. 1998, Gomez et al. 2002, Hefnawy and Tórtora-Pérez 2010,). These maladies, though not directly related, share a link through selenium and illustrate the broad effects of micronutrient deficiencies on animal physiology.

A recent article found a correlation between whole blood selenium and selenium levels in hair (Roug et al. 2015). This finding could be a mechanism behind my postulate that selenium may play a role in the loss of guard hair, often associated with the early stages of hair loss syndrome. My results that deer deficient in selenium are more likely to have brittle hair, adds to the line of thought that micronutrients play a role in HLS.

Both sucking and biting lice were found to be more abundant on does that were selenium deficient, supporting my prediction of a negative association between lice and selenium. Sucking lice feed by attaching to an animal and “sucking” the blood of their host where biting lice feed on hair, skin and skin products on the surface of their hosts. Signs of infestations from both sucking and biting lice present as skin irritation, often causing interrupted feeding and sleeping of the hosts (Holdsworth et al. 2004). Other research into HLS and the effects of lice has reported louse numbers of 133 adults and nymphs per cm^2 (Bildfell et al. 2004). It is possible for sheep to be infested with 0.5 to 1

million lice (Durden 2009). In stark contrast, only 3 animals in my study had a total louse count above 100 adults and nymphs across all regions of the body sampled. Although the relationship was not clear, my results showed a distinction between does that were selenium deficient and those that were not. Perhaps the prolonged activity of the GPx process in selenium adequate does played some role in preventing louse proliferation.

Micronutrient levels did not account for the presence of alopecia on the deer in this study. Alopecia was observed in equal numbers of recaptured treatment animals, and the novel late captures. The winter of 2014 was relatively warm with an average temperature 7°F above the average for the region (US Climate Data 2016).
Lice typically proliferate in the coldest months (Samuel and Trainer 1971) and it is possible that conditions were not sufficient for louse numbers to reach levels that would influence hair loss.

Seven of the 13 treated does returned to the wintering grounds with a fawn the following winter post treatment. There was a similar number in the reference population, which had 11 fawns to 21 does. It did not appear that instances of twining were increased with micronutrient supplementation. The reference population had 4 instances of twins where the treatment does only had a single instance of twins. Reproduction was not found to be affected by treatment with selenium. No significant difference between fawn to doe ratio was found between selenium treated does (0.61) and untreated does (0.52). The method I used to assign fawns to does could have miss-assigned twins, which would have pushed reproduction numbers away from the treatment group in favor of the reference population. My findings do not support the findings of Flueck (1994) in which a fawn to

doe ratio of 0.83 was observed in selenium treated does compared to untreated does of 0.32. However, there are several differences between my study and Flueck (1994) that could account for the discrepancy. For one, data collection for my study occurred after the devastating Rim Fire and some deer likely died; the spring composition count was 39% lower than the previous year. Moreover, my data were collected over a much shorter time-frame and my sample size was smaller than that collected by Flueck (1994). The fire, and the associated nutritional stress, may also have changed conditions for reproduction, although it is worth noting that these factors would have impacted both control and treatment groups.

For this study, blood serum copper concentrations of deer were found to be within normal limits whether sampled before or months after treatment with the copper bolus. The mean blood copper concentration was not found to differ 2-3 months after administration of the bolus at the time of final capture; this is interesting as supplementation with copper boluses is a common practice for livestock (Pugh and Baird 2012). Perhaps, the dosage of 0.4 g Cu/kg body weight was not sufficient to affect a wild ruminant, or perhaps the level increased initially then decreased before the recapture date.

The mean blood zinc concentration increased slightly, although not significantly, between capture events, but deer were not supplemented with zinc. It is worth reporting as it is a crucial micronutrient used in the immune system (Shankar and Prasad 1998) and because zinc interacts with fire (Stankov-Jovanovic et al. 2011). Depending on severity, fire can act to either remove zinc from the environment or promote its uptake by new plants (Certini 2005, Garcia-Marco and Gonzalez-Prieto 2008).

One of my initial goals was to document the progression of HLS through the winter months to draw inference to the study of HLS and relate this information to seasonal fluctuations of lice. I was successful in re-sighting 27 does of the initial 40 treated during the winter of 2014. Of the re-sighted does only 4 presented with signs of alopecia and of those that did present, did so in late April and only when assessed in hand. Because of a warm spring the study deer began migrating in late April and the determination between regular molting and hair loss was difficult. The detection of the first signs of HLS at a distance was also challenging. All of the spring-caught deer were scored on the HLS scorecard before capturing. However once in hand, the score given before capture, was generally inaccurate after thorough examination. Often small patches of heavily groomed hair or bare skin went unnoticed even at close distance. Many of the inconsistencies of judging gradations of hair loss from a distance lead me to doubt the validity of the data that had been collected when viewed from a distance. The issues of judging HLS on a gradation scale is the difficulty to assigning reliable identifiable breaks. Other studies have judged hair loss at a distance but only assessed presence or absence (Bender and Hall 2004, McCoy et al. 2014).

Hair loss syndrome occurs over a broad geographical region and it continues to be discovered in new areas suggesting that the disease might be spreading (Bildfell 2004). Within the current distribution of hair loss syndrome, selenium soil concentrations are variable like any soil nutrient (Tolu et al. 2014). If hair loss syndrome is tied to the availability of selenium in soils, I would expect to see a distribution of HLS correlated

with known distribution of low selenium in the soil, which it does or does not appear to follow.

The Rim Fire undoubtedly influenced my ability to assess HLS. Nearly the entirety of the forest within the Jawbone deer herd's distribution was burned creating a highly modified environment. Depending on intensity, fire decreases levels of environmental selenium along with other micronutrients (Smith et al. 2000). Selenium, along with other nutrients, can be lost in gaseous form from fire (Flueck and Smith-Flueck 1990, Smith et al. 2000). Even though selenium was found to be tangentially associated with hair loss, in the form of hair brittleness, the removal of what little selenium was available may have led to increased micronutrient stress and lower selenium levels in deer. Nutritional intake balanced by energy expenditure is among the most critical factors of an animal's health (Parker et al. 1996, 1999, 2009). In a post fire environment, it would make sense that energy used during foraging might be less than nutrition gained due to the sparseness of vegetation. Considering the shortage of browse vegetation after a fire, deer were likely in a diminished state at the time of sampling. Continued monitoring in the post-fire environment is an opportunity to document the fluctuations of blood micronutrient levels in a migratory deer herd in an environment that has been damaged by fire.

This study was a test of the suggestion by Foreyt (2004) that nutrition could be a cause for HLS, and research has indicated that selenium can cause reduced hair growth (Olsson 1990, Hefnawy and Tórtora-Pérez 2010). I found that fewer hair shafts broke in does with higher blood selenium concentrations even though the breaking only decreased

by a fraction of a percent. Additionally, selenium present in rumen microorganisms may be greater than that available in browse, indicating that even though selenium may be relatively rare in the environment it can be compensated by rumen function; Whanger et al. (1978) reported a 46-fold difference in the selenium concentration in rumen microbes compared to that in vegetation. Additional work on the processing of micronutrients by wild deer could help associate early onset hair loss with selenium concentration in the blood.

The response to copper supplementation did not yield results similar to those seen with selenium; in fact, copper levels decreased after treatment. In contrast, a 5% copper sulfate solution administered at a rate of 9 mg/20 kg by formula to pronghorn (*Antilocapra americana*) fawns until weaning significantly increased blood levels in pronghorn fawns when consistently applied (Miller et al. 2001). Copper levels suitable for domestic sheep range between 0.75 and 1.70 ppm (Poppenga et al. 2012). A study that looked at 6 different methods of copper administration to sheep found that blood plasma levels were not affected by any of the six methods, one of which was an oral dose (Judson et al. 1984). Deer often have higher levels of copper during winter (Zimmerman et al. 2008), but I found that copper levels were greater when deer first arrived on the wintering grounds than during spring as they were preparing to migrate back to summer range. Possible reasons for the different findings may have resulted from the low availability of winter foraging locations and limited forage availability due to the fire.

The Jawbone deer herd was found to have a mean of 1.30 ppm zinc in the blood. I found that this herd exhibited an overwinter increase in blood levels of zinc as supported

by Zimmerman (2008) who found that zinc was slightly, but not significantly, greater in summer than winter. Zimmerman (2008) also reported higher zinc levels in deer that occupied burned areas as was the case in my study. Moreover, the mean blood zinc concentration of the Jawbone deer herd is on the high end of what is typically seen in domestic sheep which are often below 1.2 ppm (Poppenga et al. 2012).

The effects the fire might have had on louse infestations remains unknown. Both sucking lice and chewing lice spend their entire lives on the hosts, so direct impacts of fire seem unlikely. However, many deer during initial capture had a fine layer of soot on their skin just below their undercoat. The soot layer might have acted as an irritant to the lice during early winter when their numbers were still small preventing proliferation much as diatomaceous earth can be used to reduce insect numbers (Mustafa et al. 2019). Previous studies have indicated that removing lice by treating deer with ivermectin early in the louse infestation cycle proved to be partially effective in preventing proliferation than when left untreated (Foreyt et al. 2004). Treatment with ivermectin reduces internal parasite load as well as allows for hair coat to regrow more quickly (Foreyt et al. 2004).

The influence of the fire cannot be know, however its effects were even across this study, all study individuals as well as the study location had the same treatment of "catastrophic fire." With respect to general condition of the heard it is possible, although speculative, that after the fire only those individuals that were fit enough to survive the event were left alive, leaving the most healthy of the herd. The animals lost to the fire could have made for variation in my samples that was not seen when using only the animals strong enough to survive. Sampling the most-fit portion of the population may be

a reason why I saw lower levels of lice, hairloss and why I saw no difference in reproduction between groups.

CONCLUSION

Results from this study offer evidence regarding the possible interactions between selenium, the onset of HLS, and the abundance of lice in mule deer. An increased propensity for guard hairs to break in does with low blood selenium coupled with increased louse numbers in does that measured as selenium deficient are possibly related to increased mortality. Although the statistical power of my analysis was low, selenium levels were associated with the breakage of guard hairs and infestation by lice. My findings may have been influenced by simultaneous treatment with selenium and copper as well as the effects of Rim Fire which are not clearly understood.

Further research should evaluate potential factors that lead to hair loss syndrome. There is a paucity of information regarding the regional fluctuations of the blood micronutrient levels of deer. Regional differences among deer herds that are affected by HLS may account for the impact that the syndrome's symptoms have. Further research into accurately mapping the normal range for blood micronutrient levels of wild deer herds could shed light onto why the deer of the Jawbone region seem to be affected by hair loss syndrome.

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APPENDIX A: DEER BODY CONDITION CRITERIA

SCORE	**WITHERS**	**RIBS**	**TAIL & HIPS**
5	Top of thoracic ridge even with loin	Discernible only at ventro-caudal extent	Sacral ridge, ilium, ischium are virtually indiscernible
4	Top of thoracic ridge extends < ¼" above loin	Mostly discernible except at dorso-cranial extent	Sacral ridge discernible from ilium ~ midway to tail base Ischium & sacro-sciatic ligament discernible
3	¼ - ½" of thoracic ridge discernible	Ribcage entirely discernible	Entire sacral ridge discernible but not prominent (<¼") Can pinch ~ ¼" of sacro-sciatic ligament on medial surface
2	⅔ - ¾" of thoracic ridge discernible	½ finger insertable between ribs at ventro-caudal extent	Sacral ridge prominent to tail Can pinch ~ ½" of sacro-sciatic ligament on medial surface
1.5	~ 1" of thoracic ridge discernible		Can pinch ~ ⅔" of sacro-sciatic ligament on medial surface
1	> 1 ¼" of thoracic ridge extending above flesh	1+ finger completely insertable between ribs at ventro-caudal extent	Can pinch ~ ¾" of sacro-sciatic ligament on medial surface Sacral ridge, ilium, ischium, tuber coxae, and sacro-sciatic ligament (entire top of rump) are prominent

0.5	Can pinch ~ 1" of sacro-sciatic ligament on medial surface

APPENDIX B: HAIR LOSS SCORE

Score	Symptoms
0	No hair loss
+	Hair loss present, but degree unknown
1	One body region with hair loss about the size of a fist
2	2 body regions with hair loss about the size of a fist **or** one region with effects on about 50% of area
3	3 body regions with hair loss about the size of a fist **or** one body region fully effected **or** 2 body regions with >50%.
4	4 body regions with hair loss about the size of a fist **or** 2 body regions fully affected **or** 1 body region fully affected and 2 body regions >50%
5	Loss of undercoat with raw skin easily seen on any body region