Effects of Mineral Supplements on California Bighorn Sheep in Northern Nevada

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Abstract: Routine capture of California bighorn sheep (*Ovis canadensis californiana*) in northwestern Nevada in 2000 resulted in a high rate of mortality. Capture and handling protocols, along with biological samples, were evaluated to discern the causative agent or process responsible for the excessive mortality. The only consistent result was low blood selenium levels, a key element in maintenance of the immune system and lactation in female sheep. From 2000 to 2004 mineral supplements were added to areas frequented by bighorns in hopes of elevating metabolic mineral levels. In 2000, pretreatment samples indicated 6 of 31 samples at or above normal liver selenium levels; however, considerable variability among animals in all areas indicated site differences with regards to geology, moisture patterns, selenium availability in soil and plants, and plant composition. No significant change was seen in mean selenium level for the control samples over the course of the study; whereas, treated units showed a significant increase from 2000 to 2004 ($t = 2.73$, $P = 0.006$). Stable or elevating lamb ratios allowed for herd growth in control units during the study. Lamb ratios in treated units were variable, with only one unit showing a significant increase. Herd performance, as measured by finite rates of increase, were strongly positive for most herds in treated or control units. Application and monitoring of mineral blocks in any given mountain range cost approximately $4,500/yr based on distributing 40 blocks at key sites. Treated units had a significant increase in mean liver selenium values, but there was no strong evidence to indicate the increase was due solely to the use of mineral supplements. Herd performance was not increased during the study, but only one herd within a treated unit showed any signs of declining numbers. If the management goal is to prevent major declines rather than produce population increases, mineral supplements may be rationalized.

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Throughout the western United States, bighorn sheep (*Ovis canadensis*) restoration efforts intensified over the last 2 decades. Costs and benefits of "putting sheep on the mountain" have been great. Each new bighorn population adds to the value of these herds as well as the growing concern for their sustainability. Epizootics in wild bighorn sheep populations occur at a tremendous cost to sportsmen and achieving the goals of restoring bighorns to historic habitats. A growing appreciation by biologists and pathologists, is that secondary stressors, both environmental and intrinsic, may be predisposing or contributing to epizootics. Ill-thrift bighorns that lack a strong and previously-challenged immune system may be at greater risk when harsh environmental conditions or intrinsic stressors occur. One of the key elements to maintaining a healthy and effective immune system to viruses and bacteria is selenium (National Research Council 1985, Berger 1993, Underwood and Suttle 2000, Hnilicka et. al. 2002). Few, if any, studies focused on measuring selenium levels in bighorn with or without mineral supplements to determine baseline selenium levels and to monitor populations relative to the importance of selenium in maintaining a healthy herd and positive population responses (survival and recruitment). Donald et al. (1993) provided selenium supplements to domestic ewes and found increased survival of newborn domestic lambs.

The impetus to evaluate the influence of selenium on maintaining healthy bighorn herds came about during a routine aerial net gun capture of California bighorn sheep (*Ovis canadensis californiana*) between January 31 and February 2, 2000, in Humboldt County, Nevada (41.65 N, 118.73 W). Five bighorn herds were captured from the Santa Rosa Range (Unit 051, North), Montana Mountains (Unit 031), Pine Forest Range (Unit 032), Bartlett Peak (Black Rock Range, Unit 034), and the Jackson Mountains (Unit 035) (Fig. 1). A high rate of mortality occurred in which 21% of 33 sheep died in the transport trailers prior to release. The capture project was stopped and potential reasons for the high mortality rate assessed, including review of the field methods, analysis of samples collected, and necropsy of 3 of the dead sheep (Sohn 2000).

Capture and handling processes were evaluated to determine if excessive stress may have led to the bighorn mortalities. Helicopter pursuit times for most of the animals were < 5 min (acceptable in comparison to previous operations in Nevada). Day time temperatures were ~13° C (elevated above typical temperatures of 4 - 10 ° C). Rectal temperatures were elevated (most were >41 ° C) compared to past net gun captures in Nevada in which only 10 – 30% are >41° C (Nevada Division of Wildlife, unpublished data). Standard preventive treatments of antibiotic (Penicillin, 5 cc), vitamin E/selenium supplement (Bo-Se, 3 cc), anthelmintic (Ivermectin, 3 cc), and clostridial vaccine (8-way, 2 cc) were given subcutaneously to each bighorn. Standard biological samples included pharyngeal and nasal swaps, fecal pellets, and blood from the jugular vein. Handling times on the ground prior to loading into trailers for a few animals was >5 min (normal is 3 – 4 min) but there was no apparent pattern between bighorns that died and time spent on the ground.

Blood samples were assessed for several disease agents including: *Brucella ovis, Leptospira interrogans,* infectious bovine rhinotracheitis virus, bovine viral diarrhea virus, bovine respiratory syncytial virus, parainfluenza-3 virus (PI-3), ovine progressive pneumonia virus, bluetongue virus, and epizootic hemorrhagic disease virus (EHD) (California Animal Health and Food Safety Lab). Only positive results are reported. Seven of 16 sheep from the Santa Rosa Range, 3 of 5 from Bartlett Peak, and 1 from Pine Forest had antibodies against PI-3. One bighorn from the Montana Range had antibodies against EHD. One sheep from each of the Montana and Bartlett Peak ranges, 3 from Pine Forest, and 2 from Jackson Mountains had antibodies against *L. interrogans.* However, the low levels suggest no ongoing problem at the time of capture.

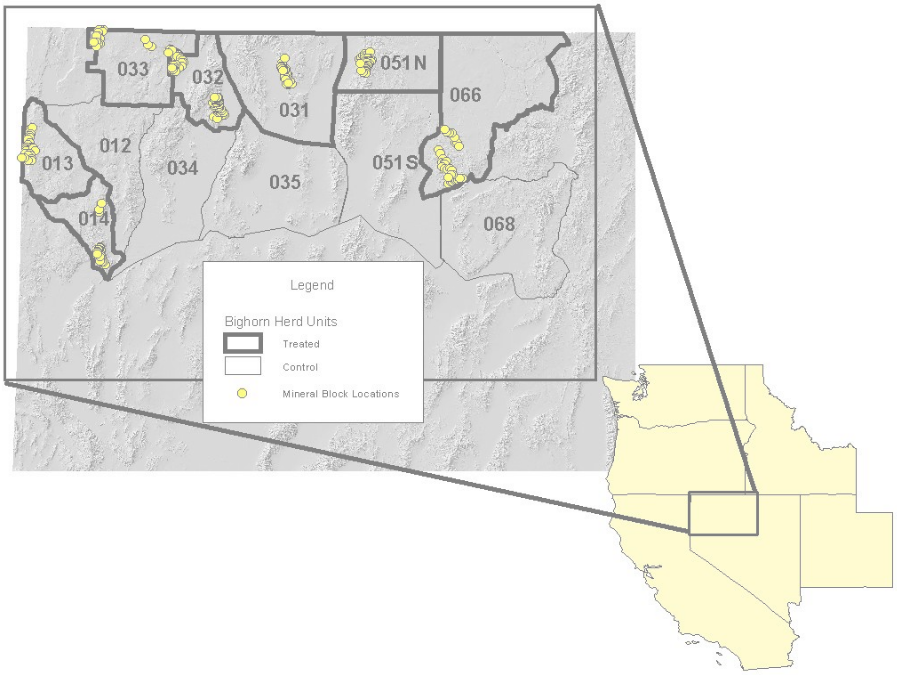

Figure 1. Geographic distribution of treated (blocks) and control (no blocks) management units in northern Nevada.

On February 15, 2000, Nevada Division of Wildlife (NDOW) biologists, pathologists, and wildlife veterinarians met to discuss future steps needed to isolate the cause of mortalities. From this meeting, a second capture was suggested to collect more biological samples, and assess general body condition and response to helicopter pursuit. In late February 2000, 21 bighorns were captured including 1 ewe that died during handling as a result of complications associated with verminous pneumonia.

Necrospy of three bighorns that died during the initial capture provided no consistent clues or pattern of mortality, in particular, no *Pasteurella* spp. or biotype of concern were isolated (Table 1). Ten nasal cultures from the second capture were negative for virus or *Chlamydia* spp. isolations. Moderate numbers of *Protostrongylus* spp. (nematode lungworms) were found in 25% of the bighorns. In 52% of the sheep low blood selenium levels occurred relative to the normal range in domestic sheep of 0.08 to 0.5 ppm. (California Animal Health and Food Safety Lab, unpublished data). This normal range of blood selenium is consistent with other research in domestic sheep (Hnilicka et al. 2002) and bighorns (Puls 1994). Selenium is a key element in maintenance of the immune system (National Research Council 1985, Berger 1993, Underwood and Suttle 2000, Hnilicka et. al. 2002) and lactation in females (Smith 1994). There was no apparent causal relationship between environmental conditions and the mortalities.

Discussions and evaluations of pertinent facts and realistic solutions continued among pathologists and veterinarians.

Table 1. *Pasteurella* spp. in pharyngeal and nasal swabs from California bighorn sheep in northern Nevada, January and February 2000.

Species	Biovariant	Beta hemolytic	# of sheep	Mountain Range
Pasteurella trehalosi	2	no	7	Santa Rosa
	2^{B}	no	7	Santa Rosa
	4	no	3	Santa Rosa
	2	no	5	Montana
	4	no	1	Montana
	2	no	3	Pine Forest
	2	no	4	Bartlett Peak
	2^{G}	yes	1	Bartlett Peak
	2	no	5	Jackson
	2^{B}	no	3	Jackson
P. haemolytica	1^{α}	yes	1	Santa Rosa
	$9^{\alpha\beta R}$	yes	1	Santa Rosa
	3	no	1	Montana
	5	no	1	Montana
	10^{α}, $10^{\alpha B}$	no	6	Montana
	1	yes	1	Pine Forest
	1^{B}	no	3	Pine Forest
	3	no	1	Pine Forest
	5	no	4	Pine Forest
	8	no	1	Pine Forest
	$9^{\alpha\beta R}$	yes	3	Pine Forest
	1^{B}	no	2	Bartlett Peak
	3^{B}	no	1	Bartlett Peak
	$9^{\alpha\beta R}$	yes	2	Bartlett Peak
	$10^{\alpha\beta E}$	no	2	Bartlett Peak
	16^{E}	yes	1	Bartlett Peak
	3	no	1	Jackson
	5	no	1	Jackson
	10	no	3	Jackson
	16^{E}	no	1	Jackson
P. multocida	*multocida b*	no	2	Bartlett Peak

Selenium levels were the only parameter with significant departure from normal levels. Therefore, we developed a study to add mineral supplements to areas frequented by bighorns in hopes of elevating metabolic mineral levels as measured from the liver of harvested animals.

Methods

The study design used California bighorn sheep herd management units as the sampling unit (Fig. 1). Biological samples (as below) from rams harvested in fall hunting seasons were used to evaluate changes in selenium levels, parasite loads, and past bouts of pneumonia within sampling units. Some management units initially identified as part of the study were eliminated due to lack of samples. The largest bighorn population, located in Unit 051, was separated into two subunits to equalize sample sizes among units. Six treated units and four control units were used in the final analyses (Table 2).

Beginning with the 2000 bighorn hunting season, all hunters were given sampling packets to collect liver, lung, and fecal samples as pre-treatment samples prior to mineral supplementation. Post-treatment samples were collected from rams harvested during the late summer of 2001, 2002, and 2004. Liver tissue was assessed for selenium levels (ppm) (standard trace element screen by California Animal Health and Food Safety Lab), fecal pellets were examined for parasite larvae (larva per gram) (modified Baermann technique by Department of Veterinary Microbiology and Pathology, Washington State University, as per Samuel and Gray 1982), and lung tissue assessed by histopathology (California Animal Health and Food Safety Lab). Trace element screening for minerals other than selenium was discontinued after 2000 due to funding concerns and fairly standard values observed. Parasitologic examination focused primarily on detecting *Protostrongylus* spp. larvae in fecal pellets, although they provide, at best, only a relative index of *Protostrongylus* organisms actually in the lungs (Festa-Bianchet 1989, Festa-Bianchet 1991). Histopathology of lung tissue was done to detect scarring indicative of previous pneumonia, evidence of verminous pneumonia, and to substantiate fecal larval identity.

During June 2001, mineral blocks were dropped from a hovering Bell Jet Ranger helicopter in remote sites within treated units. To reduce use by horses and cattle, blocks were not placed near water. Blocks consisted of 70% sodium chloride, 4% calcium, 2.5% magnesium, 0.36% sulfur, 0.24% potassium, 0.15% phosphorus, 2500 mg/lb zinc, 370 mg/lb iodine, 248 mg/lb iron, 48 mg/lb selenium, 5.8 mg/lb manganese, and cane molasses to bind the minerals and prevent leaching before animal use (Garino Livestock Supply, Chico, California). A block was placed outside the NDOW field office in Winnemucca near the study area in June 2001 to allow field

Table 2. Study design of mineral application to bighorn management units in northern Nevada, 2001 to 2004.

Treated Units		Control Units	
Unit	**Mountain Range**	**Unit**	**Mountain Range**
013	Hays Canyon Range	012	Calico Mountains
031	Montana Mountains	034	Black Rock Range
032	Pine Forest Range	035	Jackson Mountains
033	Sheldon National Wildlife Refuge	051 South	Santa Rosa Range
051 North	Santa Rosa Range		
066	Snowstorm Mountains		

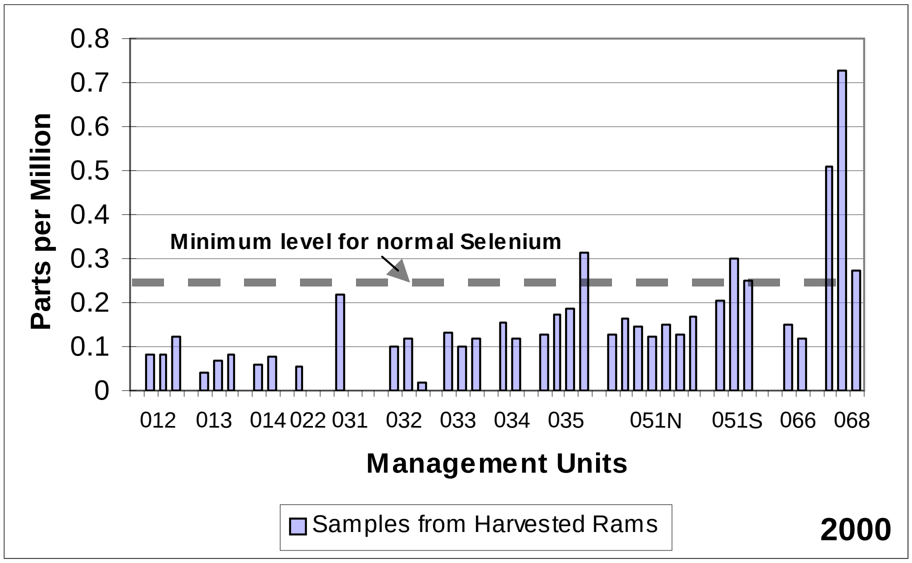

Figure 2. Pretreatment liver selenium levels from harvested California bighorn sheep in northern Nevada, August and September 2000.

personnel to account for weathering in estimating the actual animal use of blocks in treated units.

Analysis of variance (ANOVA) with significance level of $P \leq 0.05$ was used to examine the variability of liver selenium levels in treated and control units from 2000 through 2004. Students *t*-test was used to compare treated and control sample means within and among years.

Results

Annual data: Thirty-seven usable samples were collected from hunter-harvested rams during the pretreatment period in 2000 (31 samples from treated or control units). All but 6 liver samples had below normal selenium levels (normal 0.25 – 1.5 ppm based on domestic sheep; California Animal Health and Food Safety Lab, December 2000, unpublished data) (Fig. 2). Abnormal results on other trace minerals included: copper – 2 samples below normal, 13 elevated, and 1 toxic level (264 ppm) (normal 25 – 100 ppm); zinc – 7 samples below 25 ppm (normal 30 – 75 ppm); lead – 2 samples at 4 and 7 ppm (normal <1.0 ppm) (California Animal Health and Food Safety Lab, unpublished data). Six of 30 fecal samples had moderate to high lungworm larvae counts (10 – 123 larve per gram) (William Foreyt, pers. comm.). Histopathologic examination of lung tissues revealed mild verminous pneumonia in 7 samples. Lung samples from the 3 rams harvested in the southern portion of Unit 051 had mild verminous pneumonia.

Thirty-one usable liver samples were collected from hunter-harvested rams in 2001. All but 5 liver samples had below normal selenium levels (Fig. 3). Only 2 of 29 fecal samples had moderate or high lungworm larvae counts. Mild verminous pneumonia was detected in only 1 lung sample. Coincidentally, 31 usable liver samples were collected from hunter-harvested rams in 2002. All but 6 liver samples had below normal selenium levels (Fig. 4). Only 1 of 30 fecal samples had moderate or high lungworm larvae counts. A small number of *Protostrongylus* spp. larvae were found in one lung sample.

Table 3. Field assessment of mineral blocks checked in summer 2002 for use by bighorns.

Mountain Range	Unit	Total Blocks	# Checked	% Checked	% used	% moderate to high use
Hays Canyon Range	013	25	0	0	na[a]	na
Granite Range	014	20	0	0	na	na
Montana Mountains	031	30	26	87	46	19
McGee Mountain	032	30	0	0	na	na
Pine Forest Range	032	40	26	65	54	4
Sheldon NWR	033	18	0	0	na	na
Santa Rosa North	051	40	31	78	84	52
Snowstorms	066	33	8	24	88	50
TOTAL		236	91	39		

[a]na -not applicable

Table 4. Selenium (parts per million) in livers from hunter-harvested California bighorn rams in northern Nevada, 2000 to 2004.

	Treated Units			Control Units		
Year	Mean	SE	N	Mean	SE	N
2000	0.119	0.011	19	0.177	0.023	12
2001	0.144	0.015	17	0.170	0.021	10
2002	0.182	0.015	21	0.178	0.023	10
2004	0.203	0.029	18	0.168	0.049	6

Twenty-four usable liver samples were collected from hunter-harvested rams in 2004. No lungs or fecal samples were collected. Nine liver samples had normal selenium levels (Fig. 5).

Mineral block use by bighorn sheep was checked during summer 2002 in 4 mountain ranges with treated units (Table 3). A relatively high proportion of blocks were checked but estimated use was variable, particularly the number of blocks assigned to the moderate to high use category.

Treatment and year comparisons: Considerable variability in liver selenium levels was observed in samples collected in 2000, the pretreatment year, in both treated and control units ($F_{1,36}$ = 0.08, P = 0.779). Mean liver selenium levels between treated and control units in 2000 (Table 4) were significantly different (t_1 = 2.31, P = 0.017), indicating considerable site differences among and within mountain ranges. Although the pretreatment control mean was higher than the treated mean, both were below the minimum selenium standard of 0.25 ppm.

Two-way ANOVA evaluating treatment and yearly variation in mean liver selenium levels across all years of the study revealed a significant difference between treatments ($F_{1,160}$ = 17.39, P < 0.001). There was no significant change in mean selenium level in control samples over the course of the study;

however, selenium levels increased steadily in treated units (Fig. 6). Due to small sample size (n = 2 within a single unit and year) and large variability among samples within control units, statistically significant changes in mean selenium values from 2000 to 2004 in any given unit were not detected. However, increases from 2000 to 2004, some almost 2-fold, were noted in the following treated units: 031 (n = 1 for 2000; 5 for 2004), 032 (n = 3, 3), and 051 North (n = 7, 3) and in the control unit 034 (n = 2, 2) (Fig. 7).

The number of lung samples with evidence of potential verminous pneumonia declined from 2000 to 2002. Similarly, the number of moderate to high counts of *Protostrongylus* spp. larvae in fecal pellets declined from 6 of 30 samples in 2000 to 1 of 30 in 2002.

Productivity (number of lambs: 100 ewes) was assessed each year from 2000 to 2004. Control units showed either stable or elevating ratios that allowed for herd growth (Fig. 8). The ratios in treated units were variable (Fig. 9), with only Unit 032 having a significant increase in lamb ratios, but not until 2004. Most herds had positive to strong finite rates of increase in treated and control units (Table 5). Two exceptions were the treated Unit Group 066, 068 that declined 19% and the control Unit 051 South that experienced a major dieoff from late 2003 to early 2004 due to unknown causes.

Table 5. Population growth rates for bighorn sheep populations from 2000 to 2005. Animals removed or added as part of transplant program accommodated in 2005 estimates.

Treated Units			**Control Units**		
Unit	% change	annual rate	Unit	% change	annual rate
013	67	1.11	012	50	1.08
031	56	1.10	034	17	1.03
032	11	1.02	035	33	1.06
033	31	1.06	051 South	-57	0.85
051 North	10	1.02			
066, 068	-19	0.96			

Discussion

Many factors contribute to the overall health of bighorn herds. Unfortunately, often these factors are not within the control of wildlife managers, or are cost prohibitive. The impetus for this study was to see if mineral supplements, a reasonable and affordable management action, could elevate bighorn liver selenium levels, and if this would result in increased herd health and/or performance. Statistically, treated units had a significant increase in mean liver selenium values, while control units showed no change, but there was no strong evidence to indicate this increase was solely due to the use of mineral supplements.

Comparing mineral block use and changes in liver selenium during the study showed mixed results. There were only 3 units that had at least 50% of the blocks checked. The north end of Unit 051 in the Santa Rosa Range showed both moderate to high mineral block use and a dramatic increase in mean selenium levels of harvested rams during the study. The Montana Range Unit 031 had only 19% of the blocks checked with moderate to high use and only a minimal increase in liver selenium was observed. Unit 032, the Pine Forest Range had only 4% of the total blocks checked with moderate to high use yet the liver selenium levels doubled during the study. In Unit 032 the block use and selenium level relationship is confounded by the fact that McGee Mountain blocks were not checked and the harvest ram samples each year from Unit 032 included 1 or 2 rams from McGee Mountain area. Since rams are nomadic and have large home ranges, a ram harvested in one area could easily have spent considerable time elsewhere during other parts of the year. In addition, liver selenium levels in rams may not accurately reflect values in ewes and lambs in the same herd.

In determining whether mineral deficiencies prevent herd growth, there must be a measurable impact to survival fitness or performance. In most cases where lack of selenium is a major concern in management of bighorn or domestic sheep herds throughout western North America, lamb recruitment was the primary parameter affected (Donald et al. 1994, Hnilicka et al. 2002). In our study, control units had either stable or elevating lamb ratios that allowed for herd growth. Two mountain ranges had consistently low selenium levels over the course of the study (Units 012 and 013); yet reasonably good lamb production. Other mountain ranges or units had too much variability to determine whether selenium levels effect lamb production.

Costs and feasibility of conducting large-scale "on-the-ground" management can be prohibitive or impractical. We used mineral supplements because of the relatively low costs involved. Approximately $4,500/year was needed for application and monitoring of mineral blocks in any given mountain range, based on distributing 40 blocks at key sites. This includes cost of dispersing blocks, field checks on 25 to 50% of the blocks depending on topography and access to the sites, and collection of liver selenium levels from sheep, primarily rams. Monitoring must be an integral component to measure the success or failure of any health supplement program and to allow fine-tuning to improve potential success.

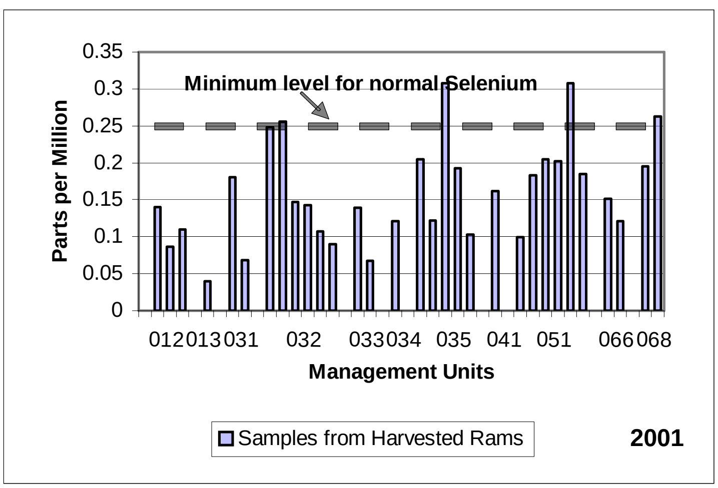

Figure 3. Liver selenium levels from harvested California bighorn sheep in northern Nevada, August and September 2001. Since mineral blocks were present only since June 2001, treated and control units were not differentiated.

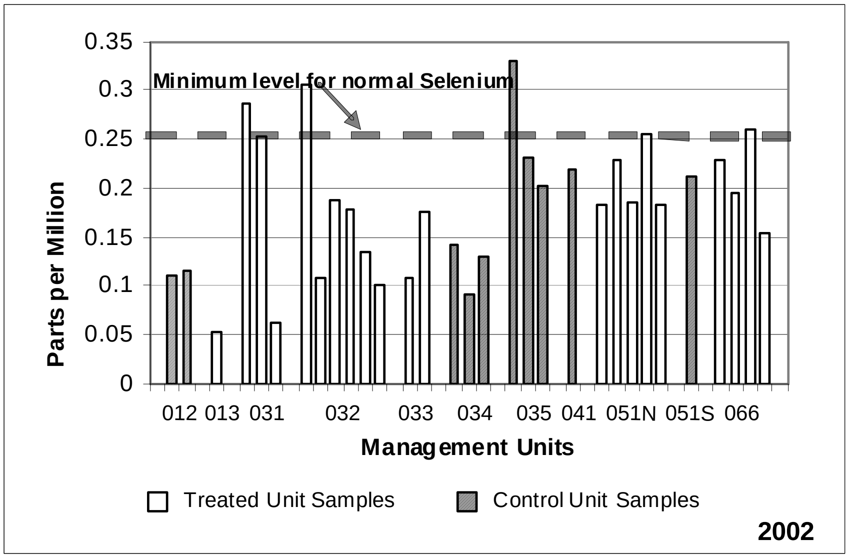

Figure 4. Liver selenium levels from harvested California bighorn sheep in northern Nevada, August and September 2002.

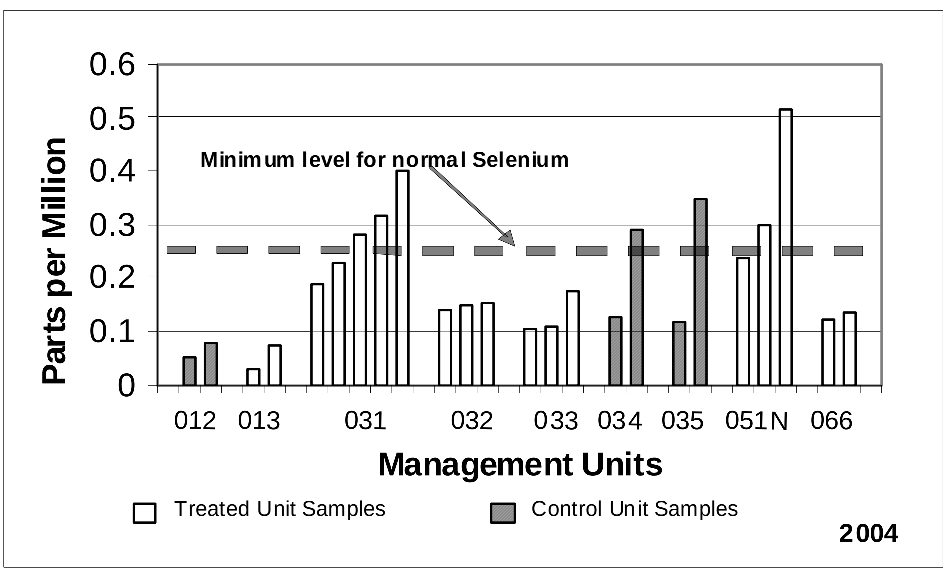

Figure 5. Liver selenium levels from harvested California bighorn sheep in northern Nevada, August and September 2004.

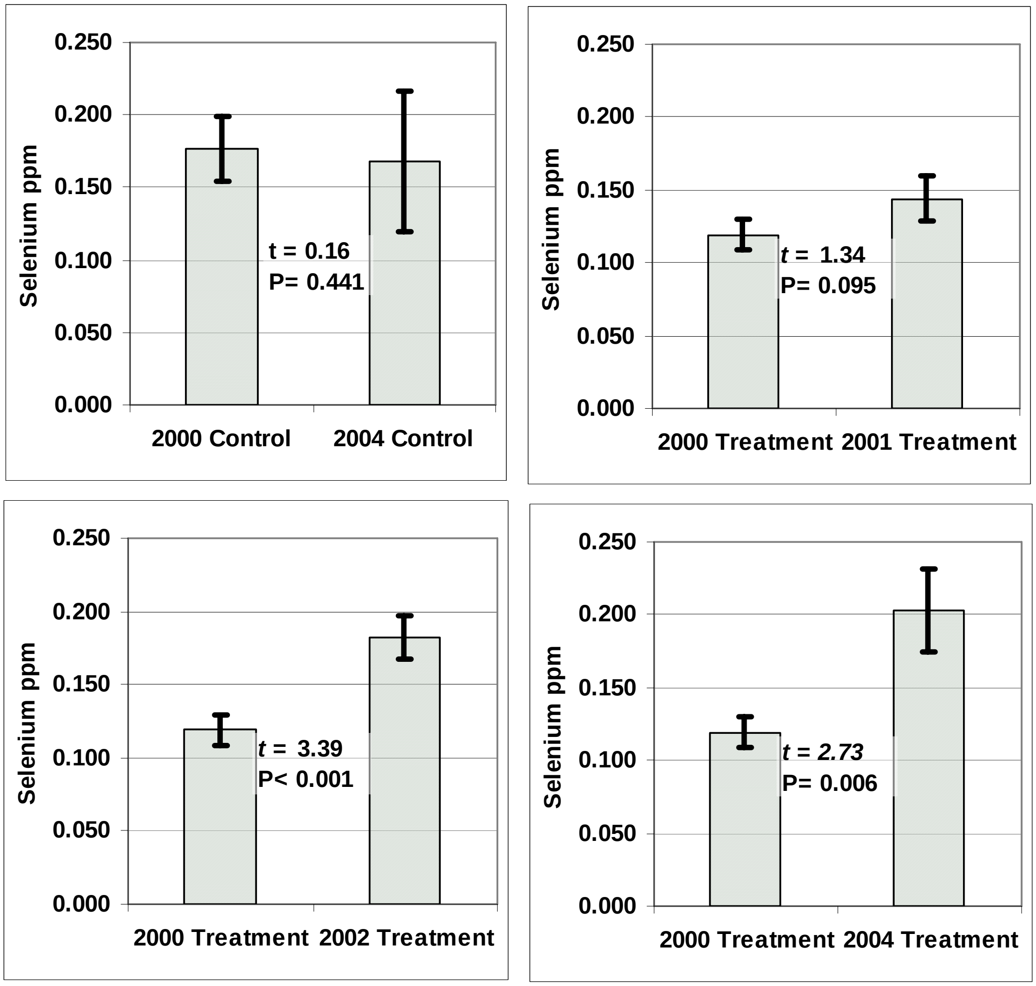

Figure 6. Comparison of yearly differences in mean liver selenium levels in California bighorns in northern Nevada.

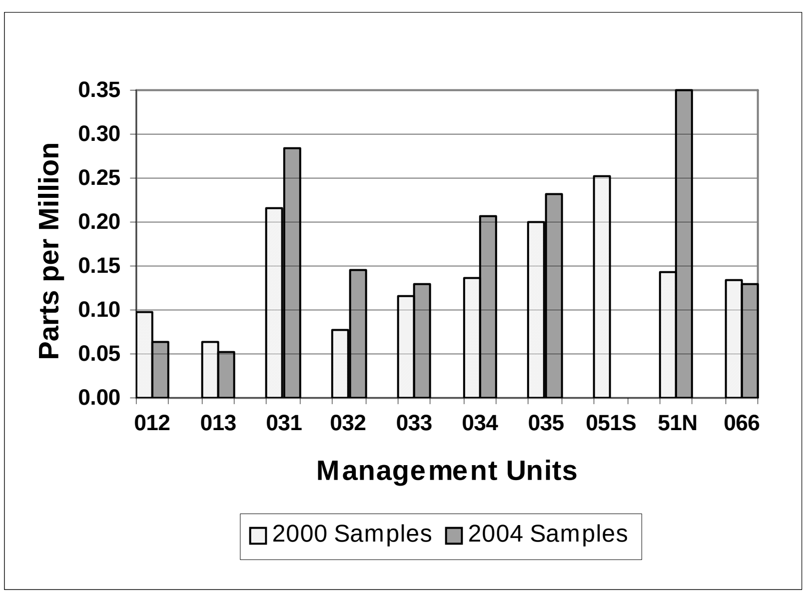

Figure 7. Comparison of mean liver selenium levels in California bighorns before (2000) and after (2004) mineral treatment in northern Nevada.

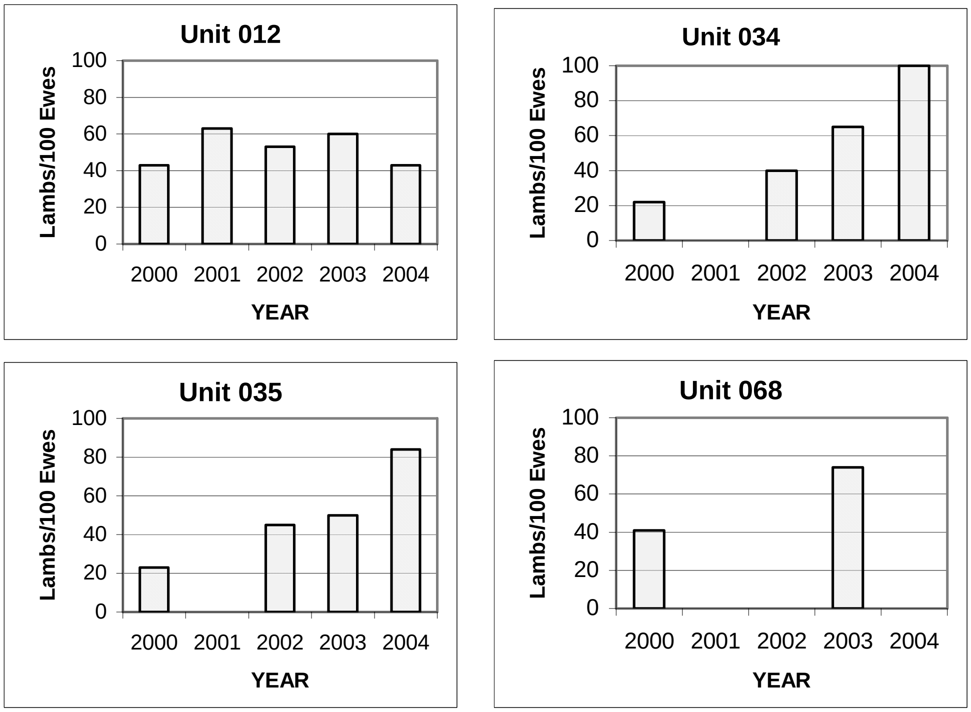

Figure 8. Lamb to ewe ratios (collected from August – November) in control units where no mineral blocks were applied.

Placement of blocks at watering and feeding sites or geographic features frequented by bighorns may enhance overall use of the blocks. But this may be off-set by blocks being used by feral horses and livestock.

If health assessments indicate bighorn immune systems are compromised by past exposure to viruses, or soil/vegetation analysis indicates significant differences in selenium availability compared to most other areas, then a mineral supplement effort may be warranted. However, use of mineral blocks simply because it is doable and relatively inexpensive compared to other more permanent or landscape-wide management actions, is not prudent.

Though the relationship of mineral use and selenium levels in the Santa Rosa Range is intriguing, no herd performance markedly increased during the study. If the management goal is to prevent major declines rather than to produce population increases, then mineral supplementation may be rational. Investigation of bighorn population declines is leading to wider acceptance that multiple stressors involving environmental, climatic, and biological variables contribute to the decline. There are many variables and conditions that contribute to major population declines. Selenium is certainly not a “silver bullet” or even “insurance” to guard against epizootics or other health-related risks to a herd. This study also contributed to establishing standard selenium levels in wild bighorn herds in the Great Basin from which to base informed decisions on the need or opportunity to enhance it.

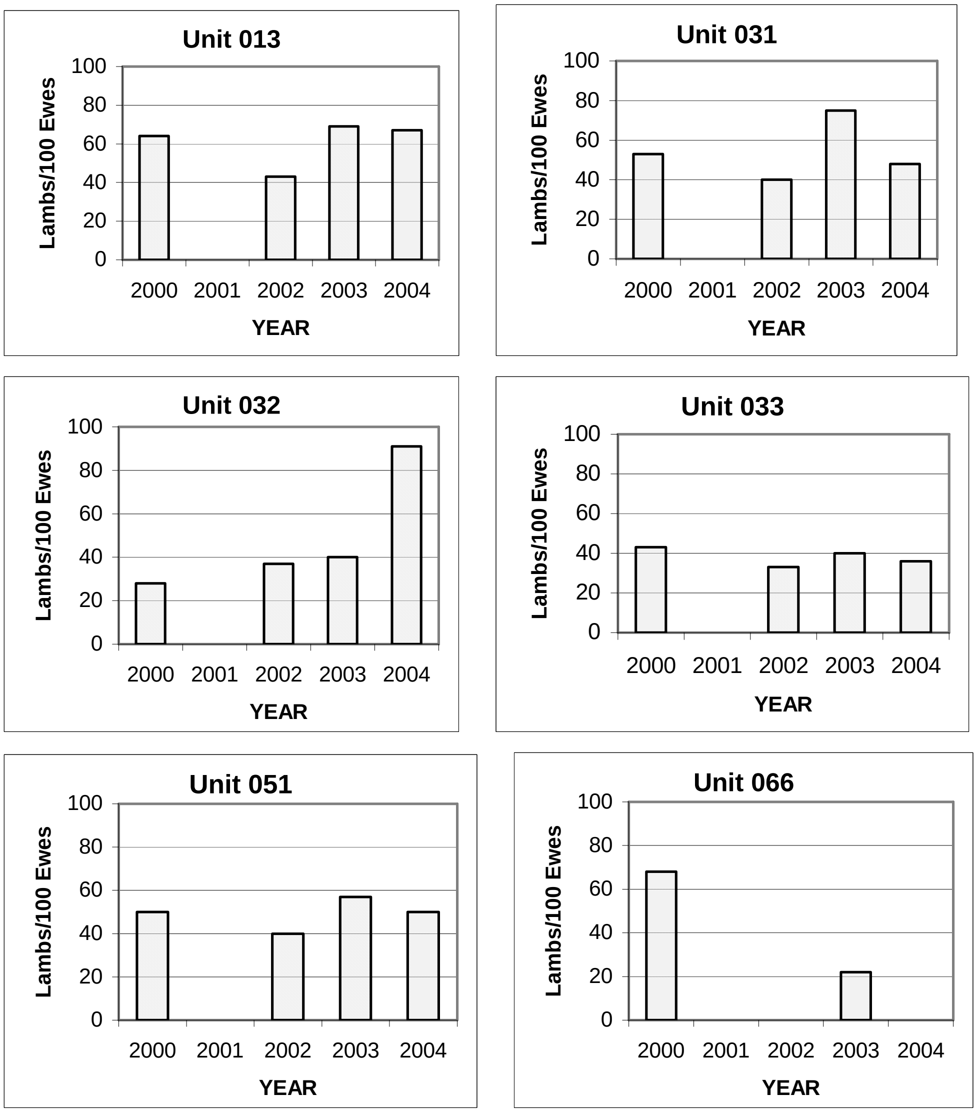

Figure 9. Lamb/ewe ratios in units treated with mineral supplements beginning June 2001.

Acknowledgments

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