

SELENIUM TOXICITY IN SHEEP GRAZING ON RECLAIMED
PHOSPHATE MINING SITES

A Thesis

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Abstract

Phosphate mining operations in southeastern Idaho have exposed selenium that was originally sequestered in the subsurface. Sheep deaths in these areas have been attributed to consumption of forage and water that is high in selenium concentration. This study was developed to monitor the response of sheep to natural exposure to selenium in mining areas. A total of 72 sheep were used (consisting of 24 ewes and their lambs) and were divided into three treatment groups. The control (Con) group was exposed to trace selenium in both the forage (0.04 – 0.20 ppm Se dw) and water (1.65 ppb Se). The low selenium (LoSe) group was exposed to elevated levels of selenium in forage (0.05 – 13.00 ppm Se dw) and trace concentrations of selenium in water (1.65 ppb Se). The high selenium (HiSe) group was exposed to elevated selenium in both the forage (0.33 – 110.00 ppm Se dw) and water (340 – 415 ppb Se). The study was constructed in three phases. The first phase was the baseline phase, in which all sheep were grazed on normal (low levels) selenium forage and water for three weeks. The exposure phase followed when the LoSe and HiSe sheep were exposed to elevated selenium levels on reclaimed mine sites for four weeks. The depuration phase was the last phase when sheep were again grazed on normal selenium forage and water for approximately two weeks. Blood, serum, fecal, urine, plant, soil and water samples were collected weekly for selenium analysis. Blood and serum selenium levels increased during the exposure phase of the study to levels of 1.27 to 1.36 ppm selenium respectively in the HiSe group. Serum selenium levels in this group returned to baseline values during the depuration phase. Blood selenium levels also declined during the depuration phase but at a slower rate. A serochemistry panel and complete blood counts were performed. Necropsies were also conducted on one ewe and lamb from the HiSe and Con groups after the exposure

phase. These sheep were examined for gross and histopathological lesions examination in the liver, kidney, skeletal muscle, heart, spleen and lung. Sheep in the HiSe group attained tissue levels of selenium that would be consistent with exposure to toxic levels. However, only one sheep died of selenium toxicity in the HiSe group. Sheep in the HiSe group also had a slight decrease in body conditioning most likely due to an early aversion to feed and water with elevated levels of selenium. No other illnesses or signs of selenium toxicity were observed in the remaining sheep during the exposure phase of the study. Sheep mortality is expected to occur in these areas, and varies year to year and may be result from a combination of selenium exposure, environment stress and management practices.

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Table of Contents

Authorization to Submit Thesis.....	ii
Abstract.....	iii
Acknowledgements	v
Table of Contents.....	vi
List of Figures.....	viii
List of Tables	x
1.0 Introduction.....	1
2.0 A Brief Overview of Selenium and Objectives	3
2.1 Selenium Chemistry and Forms in the Environment.....	3
2.2 Selenium Accumulation and Toxicity in Soils and Plants.....	4
2.3 Selenium Accumulation in Water.....	8
2.4 The History of Selenium Toxicity in Domestic Livestock	10
2.5 Selenium Deficiency.....	16
2.6 Absorption, Metabolism and Excretion of Selenium in Domestic Livestock	19
2.7 Selenium Toxicity: Biochemical Functions and Mode of Action	21
2.8 Experimental Objectives/Rationale	24
3.0 Materials and Methods.....	25
3.1 Experimental Design.....	25
3.2 Blood Analysis.....	27
3.3 Fecal and Urine Analysis.....	28
3.4 Water Analysis.....	29
3.5 Plant Analysis	29

3.6 Soil Analysis	30
3.7 Necropsies.....	30
3.8 Statistical Analysis.....	30
4.0 Results	32
4.1 Total Selenium in Blood and Serum.....	32
4.2 Clinical Chemistry	34
4.3 Pregnancy Report.....	37
4.4 Necropsy and Histopathology.....	37
4.5 Total Selenium in Urine and Feces.....	38
4.6 Total Selenium in Water	39
4.7 Total Selenium in Plants	41
4.8 Total Selenium in Soils.....	42
4.9 Grazing Observations.....	43
5.0 Discussion.....	44
6.0 References.....	54
7.0 Appendix.....	62
7.1 Serochemistries within the Normal Reference Range	63
7.2 Serochemistries Above the Normal Reference Range.....	80
7.3 Serochemistries Below the Normal Reference Range	83
8.0 Appendix.....	87
8.1 Animal Care and Use Committee	88

List of Figures

Figure 2.1.1 Selenium cycle in nature	5
Figure 2.2.1 Selenomethionine biosynthesis in plants.....	9
Figure 2.6.1 Metabolism of selenium in animals.....	22
Figure 2.7.1 Reduction of H ₂ O ₂ (lipid hydroperoxides or sterol hydroperoxides) by glutathione peroxidase and equivalents of reduced glutathione (GSH).....	24
Figure 4.1.1 Total whole blood selenium	33
Figure 4.1.2 Total blood serum selenium	34
Figure 4.5.1 Total selenium levels in sheep urine	40
Figure 4.5.2 Total selenium levels in sheep feces	40
Figure 7.1.1 Serum chloride levels	63
Figure 7.1.2 Serum potassium levels	65
Figure 7.1.3 Serum sodium levels.....	66
Figure 7.1.4 Sodium eosinophil numbers	67
Figure 7.1.5 Hemoglobin levels.....	68
Figure 7.1.6 Monocyte numbers	69
Figure 7.1.7 Platelet numbers	71
Figure 7.1.8 Red blood cell numbers	72
Figure 7.1.9 White blood cell numbers.....	73
Figure 7.1.10 Serum creatinine levels.....	74
Figure 7.1.11 Serum urea levels	75
Figure 7.1.12 Total protein levels.....	76
Figure 7.1.13 Serum alkaline phosphatase levels	77

Figure 7.1.14 Serum alanine aminotransferase levels	78
Figure 7.1.15 Total bilirubin levels.....	79
Figure 7.2.1 Serum calcium levels.....	81
Figure 7.2.2 Hematocrit numbers	82
Figure 7.2.3 Serum globulin levels.....	83
Figure 7.2.4 Serum aspartate aminotransferase levels.....	84
Figure 7.3.1 Serum albumin levels	85
Figure 7.3.2 Total carbon dioxide levels	86

List of Tables

Table 2.2.1	Selenium values in various geological materials.....	6
Table 2.4.1	Selenium requirements in animals and humans.....	11
Table 4.1.1	Level of significance of whole blood selenium	33
Table 4.1.2	Level of significance of blood serum selenium	35
Table 4.4.1	Diagnostic reference ranges of selenium in sheep tissues	38
Table 4.4.2	Selenium tissue levels in the sheep that died during exposure and sheep that were necropsied after the exposure phase.....	39
Table 4.6.1	Mean selenium concentrations in drinking water	41
Table 4.7.1	Dietary selenium reference levels in a sheep.....	41
Table 4.7.2	Mean and range selenium values in major plant species	42
Table 4.8.1	Mean and range selenium levels in soil	42
Table 7.1.1	Level of significance of serum chloride levels	64
Table 7.1.2	Level of significance of serum potassium levels	65
Table 7.1.3	Level of significance of serum sodium levels	66
Table 7.1.4	Level of significance of serum eosinophil numbers	67
Table 7.1.5	Level of significance of hemoglobin levels.....	68
Table 7.1.6	Level of significance of monocyte numbers.....	70
Table 7.1.7	Level of significance of platelet numbers.....	71
Table 7.1.8	Level of significance of red blood cell numbers.....	72
Table 7.1.9	Level of significance of white blood cell numbers.....	73
Table 7.1.10	Level of significance of serum creatinine levels	74
Table 7.1.11	Level of significance of serum urea levels	75
Table 7.1.12	Level of significance of total protein levels.....	76

Table 7.1.13	Level of significance of serum alkaline phosphatase levels	77
Table 7.1.14	Level of significance of serum alanine aminotransferase levels	78
Table 7.1.15	Level of significance of total bilirubin levels	80
Table 7.2.1	Level of significance of serum calcium levels.....	81
Table 7.2.2	Level of significance of hematocrit numbers	82
Table 7.2.3	Level of significance of serum globulin levels.....	83
Table 7.2.4	Level of significance of serum aspartate aminotransferase levels.....	84
Table 7.3.1	Level of significance of serum albumin levels	85
Table 7.3.2	Level of significance of total carbon dioxide levels.....	86

1.0 Introduction

Idaho mined phosphate accounts for as much as 14 percent of the total phosphate produced yearly in the United States. The richest phosphate deposits in the western United States are found in Idaho (Hughes, 1999). Major phosphate mines in this area are open pit or contour strip operations that were developed near surface exposures of the Phosphoria Formation (Piper et al., 2000; Idaho DEQ, 2002). Phosphate mining operations in southeastern Idaho have exposed seleniferous minerals that were originally sequestered in the subsurface. The selenium exposed from mining operations has been mobilized and distributed in the soil, water and vegetation of the region due to leaching from waste rock impoundments (Mars and Crowley, 2003). Domestic livestock (equine, bovine and ovine) illnesses or deaths have been attributed to excess selenium exposure in the area.

Waste rock from phosphate mining is composed of large piles of overburden and underburden materials (i.e., minerals such as selenium) often deposited on the surface (Idaho DEQ, 2002). Rain and weathering processes result in selenium being released from waste rocks into streams and reservoirs through precipitation runoff (Lemly, 1997; Dhillon and Dhillon, 2003). Areas downstream from known seleniferous areas may accumulate selenium. This may lead to toxic conditions for grazing animals in the area. Selenium toxicity in animals may occur from consumption of selenium accumulator plant species and/or high selenium levels in water.

In 1996, six horses pastured downstream from an inactive phosphate mine were diagnosed with chronic selenosis. Additional horses pastured on a second phosphate mine property were diagnosed with selenosis in 1997 (Montgomery Watson, 1999). More than 500 sheep deaths are thought to be related to selenium poisoning in this area since the fall of

1999. Subsequently, the U.S. Forest Service notified phosphate mining companies in southeastern Idaho that elevated levels of selenium in soil, water and vegetation have been detected on and near phosphate mine sites (Petrun, 1999). Mining companies have cooperated with federal and state agencies in an attempt to manage the problem of elevated selenium levels in response to this notice. Several monitoring studies have since been conducted in birds, fish, elk and cattle. This project extends the previous and ongoing studies to include selenium exposure in sheep under semi-controlled conditions in natural settings.

2.0 A Brief Overview of Selenium and Objectives

2.1 Selenium Chemistry and Forms in the Environment

Selenium was first discovered by the Swedish scientist Jon Jakob Berzelius in 1818 and is derived from the Greek word meaning “moon” (National Research Council, 1980; Wilber, 1980). Selenium is unevenly distributed throughout the earth’s crust, averaging approximately 0.08 ppm selenium (Merian, 1984). Selenium is found in a variety of rocks, minerals, fossil fuels, soils, plants and water (Oldfield, 1974; National Research Council, 1980).

Disintegration of rocks by environmental factors result in soils of diverse characteristics (Johnson, 1975). Selenium levels within a given area vary depending upon climate, soil pH and rock development. Selenium exists in the environment in -2, 0, +4 and +6 oxidation states. Elemental selenium (Se^0) exists as an insoluble, amorphous or crystalline form. Selenide compounds (Se^{-2}) occur as various methylated and metallic selenides or as hydrogen selenide. Both of these elements are insoluble and biologically inert when combined with other metals. Selenite (Se^{+4}) and selenate (Se^{+6}) exist in the environment as oxy-anion salts or in their ionized form in aqueous environments (Wade et al., 1993; Raisbeck, 2000; Frankenberger and Arshad, 2001).

Selenium is located between the metals tellurium and polonium and the nonmetals oxygen and sulfur in the periodic table. Selenium has chemical properties that resemble sulfur and is classified as a non-metal. Selenium has an atomic number of 34 and atomic weight of 78.96 (McDowell, 1992; National Research Council, 1980). The United States produces approximately 200 tons and imports an additional 450 tons of selenium annually. Canada, Bolivia, Morocco, Russia and China are considered the primary producers of

selenium (Merian, 1984; Jansinski, 1997). The primary uses of selenium typically involve the incorporation into many metal and non-metal substances such as photocopiers and photographic toner, ceramics, rubber, glass (de-colorizes glass and makes rube colored glasses and enamels) and as an additive in stainless steel (Windholz, 1976; Wade et al., 1993).

Weathering processes release selenium from selenium-rich rocks into the soil (Bauer, 1997; Piper, 2000). Plants accumulate selenium from the soil into their tissues which may be consumed by animals and humans (Figure 2.1.1). However, selenium in soils is oxidized into selenates under alkaline and well aerated conditions. Selenates are very stable in well oxidized environments and in water are highly soluble as selenate salts. The aqueous form of selenium is freely available to both aquatic animals and plants. However, selenites are favored under mildly oxidizing conditions. Selenites are less water soluble than selenates and are largely unavailable to plants in this form (Spallholz, 1994; Dhillon and Dhillon, 2003).

2.2 Selenium Accumulation and Toxicity in Soils and Plants

Soil selenium surveys conducted in the United States have revealed soils containing as much as 90 ppm selenium, and non-seleniferous soils containing less than 2 ppm selenium (Davis et al., 2001). Seleniferous or toxic areas may be found in arid or semi-arid climates (< 20 inches in annual precipitation), soils with high pH levels (> 7.0) and soils from shale (Davis et al., 2001). Soil from shale have been found to contain the highest concentrations of selenium and is the principal source of toxic levels of selenium in soils in the Great Plains

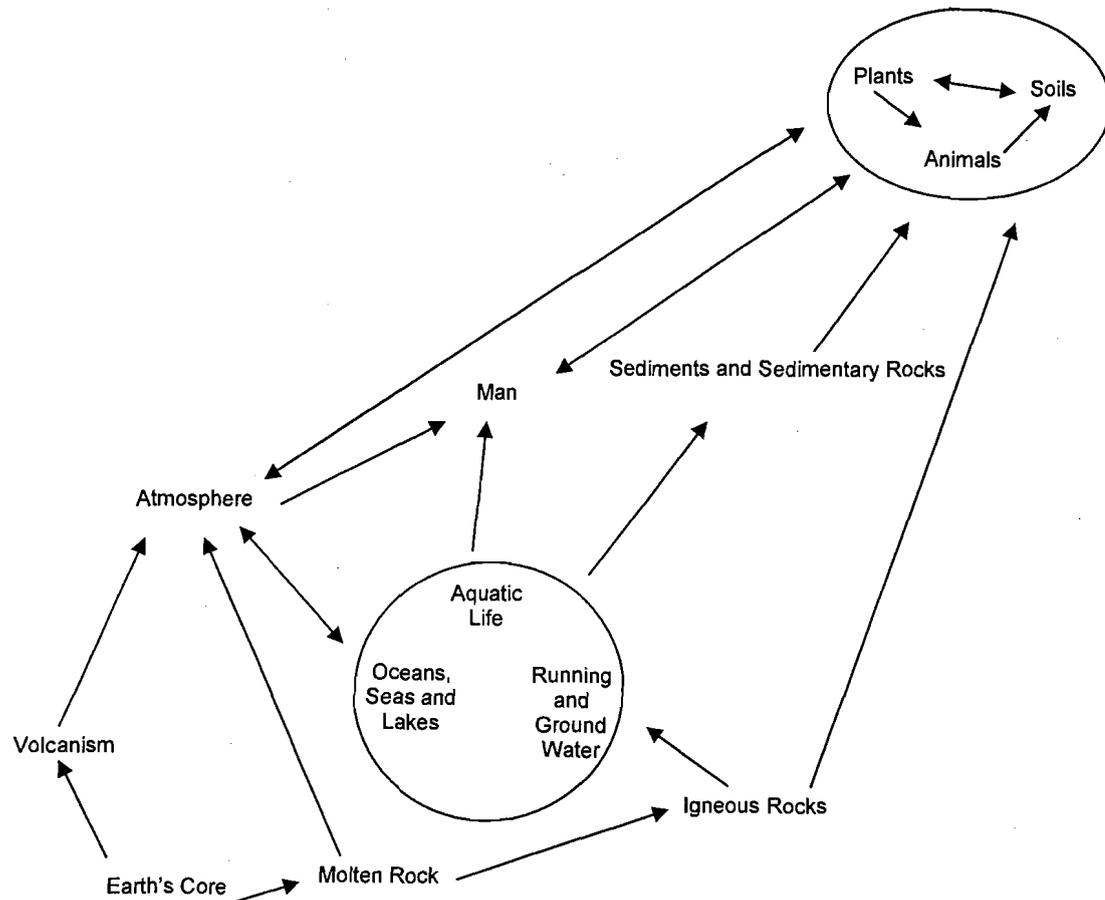


Figure 2.1.1 *Selenium cycle in nature.*
Adapted from National Research Council, 1980.

and Rocky Mountain foothills of the United States (Johnson, 1975; Fang et al., 2003) (Table 2.2.1). Certain mineral formations contain more selenium than others within the earth's crust. The highest concentrations of selenium are typically found within the Phosphoria Formation, a marine sedimentary deposit from the Permian age, from where phosphate ore is primarily mined (Petrun, 1999; Piper et al., 2000). The mining process disrupts the earth's crust and exposes selenium that had been sequestered deep within the soil to the surface environment where it is then susceptible to erosion and weathering. Potentially toxic levels of selenium in plants, soils and water may be found near or on phosphate mining sites such as

Table 2.2.1 *Selenium values in various geological materials.*
Adapted from Wilber, 1980.

Material	Selenium (ppm)
Igneous rocks	0.05
Shales	0 – 0.6
Sandstones	0 – 0.05
Limestones	0.08
Fresh water	0.02
Seawater	0.00009
Soils	0.2

in southeastern Idaho. Selenium levels as low as 0.5 mg/kg in soil and 5 mg/kg in vegetation are considered toxic levels in nature (Hamilton and Beath, 1963; Glenn et al., 1964a).

Plant uptake of selenium varies with soil pH, and is highest under alkaline soil conditions. The poorest utilization of soil selenium occurs in acidic to neutral soils where selenite and reduced forms of selenium are favored (Combs and Combs, 1986). However, it has been reported that nearly all plants growing on seleniferous soils in a water soluble or available form will absorb, metabolize and store selenium in their tissues in variable quantities (Hamilton and Beath, 1963). Yet, selenium in soils is best absorbed by plants as selenates and to a lesser degree as selenites. Selenate does not readily form complexes with minerals and is more water soluble, allowing it to be more mobile and available for plant uptake (Wade et al., 1993; Spallholz, 1994).

Other factors influencing plant selenium uptake is the species of plant, stage of growth and season (Lakin, 1972). It has been reported that plant species vary in their levels of selenium accumulation. A study conducted in the 1960's divided plants into three groups based upon their selenium accumulation capacity in seleniferous soils. Plants in the first group are called primary indicators. Primary indicators include the species *Astragalus*, *Machaeranthera*, *Haplopappus*, and *Stanleya*. These indicator plants may accumulate

selenium at very high levels, often reaching several thousand parts per million dry weight (ppm dw). Group two plants are called secondary indicators and include *Aster*, *Atriplex*, *Castilleja*, *Grindelia*, *Gutierrezia*, and *Mentzelia* species. These plants typically accumulate selenium at levels reaching 25-100 ppm dw. Primary and secondary accumulator plants are reported to be unpalatable to livestock and therefore avoided. Plants in group three include multiple grain, grass and weed species (e.g., *Agropyron*, *Medicago*, and *Hordeum*). These plants reportedly do not accumulate selenium in excess of 50 ppm dw (Rosenfeld and Beath, 1964; Harr and Muth, 1972; National Research Council, 1980; Wilber, 1980; Combs and Combs, 1986). However, it has been reported that when sufficient available selenium (i.e., water soluble selenate) occurs all species of plants will take up selenium in concentrations potentially harmful to animals (Hamilton and Beath, 1963; Larkin, 1972).

The selenium content in younger plants has been reported to be higher than mature plants. As a plant matures, nutrients are redistributed from the leaves to the root system, exposing animals to lower selenium concentrations (Lyons et al., 1995; Lyons et al., 1996). The season can also affect selenium accumulation in plants. Dry grazing seasons result in loss of moisture from soils and plant roots growing deeper for moisture. This growth may result in higher selenium concentrations in plant tissues from high selenium concentrations typically found in the subsoil (Davis et al., 2002).

Selenium accumulator plants incorporate selenium into non-proteinaceous amino acids such as selenium-methylselenocysteine, selenocystathione, selenocystine and selenohomocysteine. These plants volatilize selenium compounds at both high and low absorption levels. An offensive odor is produced by selenium accumulator plants that may be attributed to dimethyldiselenide (National Research Council, 1980). Group three

accumulators also produce dimethylselenide when subjected to moderate levels of soil selenium. However, the major selenium component within these plants appears to be the proteinaceous amino acid selenomethionine (Spallholz, 1994) (Figure 2.2.2). The absorbed amino acid readily incorporates into organic compounds such as amino acid derivatives, selenium-methylselenomethionine and selenium-methylselenocysteine, that are readily taken up by plants. For example, cereals and forage crops can convert selenium mainly into selenomethionine, which then can be incorporated into protein in place of methionine. The replacement of selenium (selenomethionine) into an amino acid sequence may not significantly alter the protein structure but may influence the activity of enzymes if the selenium incorporation occurs at an active site (Schrauzer, 2000). These substitutions for amino acids within plant proteins can cause conformational and functional changes that may result in toxicity to animals after ingestion of seleniferous plants (Schrauzer, 2000; Wright et al., 2002).

2.3 Selenium Accumulation in Water

Selenium enters water as soluble selenites and selenates. The bioavailability of selenium in water is affected by a number of environmental variables including pH, salinity, hardness, chemical forms of selenium and the presence of other chemical constituents such as sulfate ions (Dhillon and Dhillon, 2003). The greatest concentration of selenium is found within water systems that drain seleniferous soils (Combs and Combs, 1986; Dhillon and Dhillon, 2003). Higher concentrations of selenium (1-5 ppb) may result in bioaccumulation of selenium within aquatic food chains. Selenium is then likely to become a concentrated

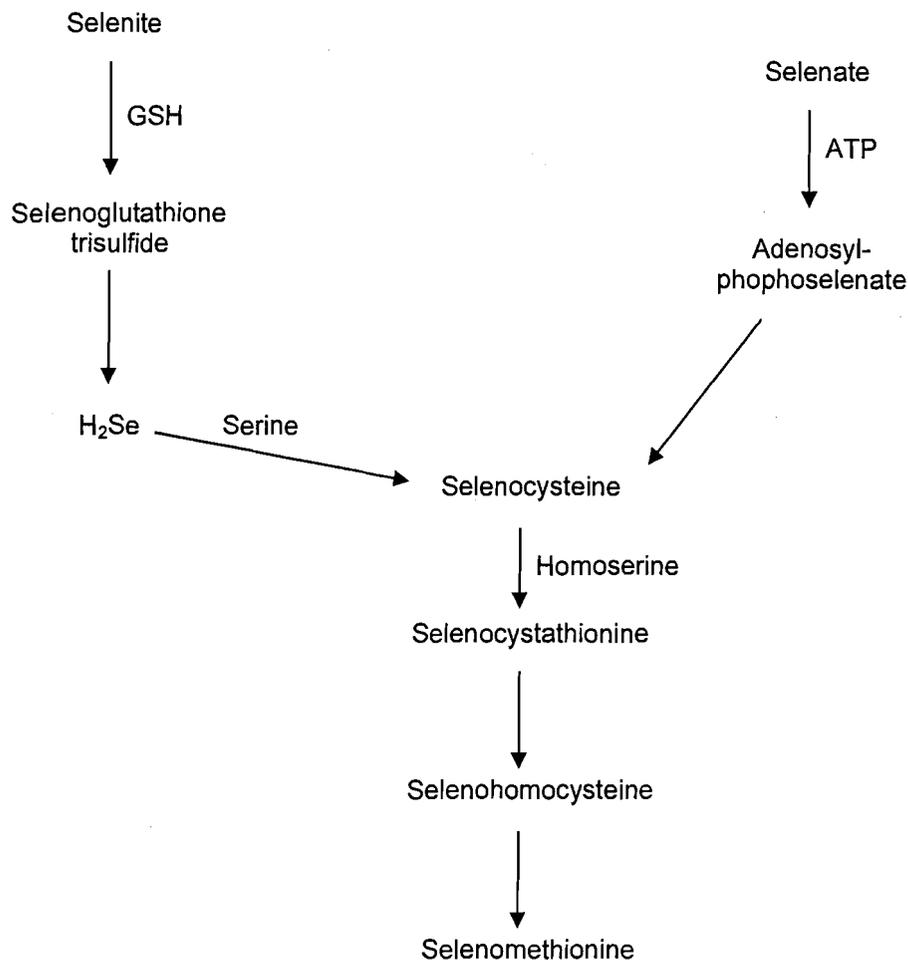


Figure 2.2.1 *Selenomethionine biosynthesis in plants.*
Adapted from Tinggi, 2003.

dietary source that is often highly toxic to fish, wildlife and domestic livestock. Selenite seems to be the predominant form of selenium in most freshwater environments (Wu et al., 1991; Lemly, 1997; Dhillon and Dhillon, 2003). Fortunately, most water systems contain concentrations of selenium at very low levels. Waters from these systems typically do not drain seleniferous soils, and for those that do, a substantial amount of selenium is removed from the water through precipitation with basic oxides such as hydrous ferric oxide (Combs and Combs, 1986).

Selenium is found in drinking water as a minor constituent, and typically occurs at concentrations from 0.1 to 100 ppb (National Research Council, 1980; Dhillon and Dhillon, 2003). The United States Department of Health, Education and Welfare set an upper limit for selenium in drinking water at 10 ppb in 1962. Extensive surveys were conducted from 1959 to 1962 on major watersheds within the United States. Results from these studies found only two samples that contained selenium levels equal to or above 10 ppb. These samples were collected from the Animas River at Cedar Hill, New Mexico (10 ppb) and the Missouri River at St. Louis, Missouri (14 ppb) (National Research Council, 1980). A later study found that the public water supplies of Jade Hills and Red Butte, Wyoming, and Grants, New Mexico contained elevated selenium levels of 174-202, 363-560, and 26-1800 ppb respectively (Combs and Combs, 1986). In these cases, selenium entered the water systems by draining through highly seleniferous soils. Another extensive study conducted in the 1960's investigated selenium levels in the major oceans. The results showed that the major oceans had selenium levels that averaged 0.090 ppb. These low levels of selenium are attributed to the precipitation of selenium under oceanic conditions with metal oxides (National Research Council, 1980).

2.4 The History of Selenium Toxicity in Domestic Livestock

Selenium is an essential trace mineral required by both humans and animals in small quantities for survival (Oldfield, 2001). Animals require selenium for cardiac and skeletal muscle growth, fertility and prevention of diseases (Sheep Production Handbook, 1997). Daily selenium requirements vary greatly for humans and selected animals (Table 2.4.1). However, it is only beneficial within a very narrow dose range for all species. Selenium

toxicity occurs in livestock and humans at levels above the normal dose range and is related to several factors including diet, gender, animal species, chemical form, dose and previous exposure to the element (Glenn et al., 1964b; Combs and Combs, 1986; Wright et al., 2002).

Selenium deficiency occurs at levels below the normal dose range (Banuelos, 1990).

Table 2.4.1 *Selenium Requirements in Animals and Humans.*
Adapted from McDowell, 1992.

Species	Daily Requirement
Beef Cattle	0.20 mg/kg
Dairy Cattle	0.30 mg/kg
Sheep	0.10-0.20 mg/kg
Horses	0.10 mg/kg
Swine	0.10-0.30 mg/kg
Dogs	0.11 mg/kg
Rats	0.11 mg/kg
Humans	50-75 µg/day

The earliest report of selenium toxicity in animals was by Marco Polo. He described a necrotic hoof disease and hair loss of horses during the thirteenth-century in China after consumption of a poisonous plant indigenous to that region (Spallholz, 1994). Chronic selenosis was later reported in the North and South Americas in livestock. The first case of human chronic selenosis, causing symptoms of hair and nail loss, was reported in Columbia during the 1560's by Father Pedro Simon (National Research Council, 1980; Rosenfeld and Beath, 1964). Chronic selenium toxicity in horses may also have caused a delay of the cavalry scheduled to relieve General Custer at the Battle of Little Big Horn. A later report by army surgeon T.D. Madison described the signs of a fatal disease that affected cavalry horses at Fort Randall, South Dakota in 1857 (Rosenfeld and Beath, 1964). Settlers also reported similar disorders in livestock during the 1890's in regions of northern Nebraska and South Dakota (Combs and Combs, 1986). Other incidences of acute poisoning in livestock were reported throughout Utah and Nebraska. It was later reported that selenium will accumulate

in several plant species that have the ability to incorporate selenium from soils.

Experimental evidence showed that selenium indicator plants required selenium for their growth and development (Harr and Muth, 1973). Finally, in the late 1950's, selenium was reported to have a protective effect against exudative diathesis in chicks and white muscle disease in livestock (reviewed by Rosenfeld and Beath, 1964; Harr and Muth, 1973; Spallholz, 1994; O'Toole and Raisbeck, 1995).

Three types of selenium toxicity (acute, subacute and chronic) in domestic livestock have been classified and described by Rosenfeld and Beath (1964). Acute toxicity occurs from the consumption of large doses of selenium within a short period of time with severe signs of toxicity and rapid onset. Death typically occurs within hours of exposure. The cause of death is likely due to selenium accumulation in the body to concentrations that overload the tissues and result in death caused by cardiac or respiratory failure (Glenn et al., 1964c; Gabbedy, 1970; Raisbeck, 2000). The highest selenium concentrations are typically found within the liver, blood, kidney and spleen in most domestic livestock. The muscle, hide, hair and bones contain only traces of selenium (Rosenfeld and Beath, 1964; Glenn et al., 1964a; Ullrey, 1987). Gross pathology in cattle and sheep manifest as petechial hemorrhages of the endocardium in the heart; acute congestion and diffuse hemorrhages in the lungs; passive congestion, hemorrhages, parenchymatous degeneration with focal necrosis in the liver; and parenchymatous degeneration and hemorrhages with nephritis in the kidney. The first signs of acute selenium poisoning typically include anoxeria and mild depression (Glenn et al., 1964c). Signs in sheep and cattle that precede death include abnormal movements (staggering), dark watery diarrhea, elevated temperatures, weak and rapid pulse, labored respiration, bloating and abdominal pain, and pupil dilation (Fishbein,

1977). The major excretory product of selenium exposure found in acute cases is dimethyl selenide (Diplock and Lucy, 1973). It has been shown that both selenite and selenate produce similar acute toxic effects (Koller and Exon, 1986). There is no treatment for acute toxicosis and death usually occurs before the disease is diagnosed (Rosenfeld and Beath, 1964; Gardiner, 1966).

Subacute toxicity results from exposure to large doses of selenium over a longer period of time and manifests as neurological signs such as blindness and disorientation and respiratory distress (Combs and Combs, 1986). This disorder may be caused by the ingestion of organic selenium compounds from native selenium indicator plants. Signs in the early stages include random wandering, circling, and stumbling over objects. Also, the animal shows little desire to eat or drink. In the later stages, the animals become ataxic and the tongue and the muscles of the throat become partially or totally paralyzed and the animals may have accelerated and labored respiration (Fishbein, 1977). Evidence of severe abdominal pain and swollen eyelids has also been reported. Death typically occurs within hours. Recovery from the early stages is possible but progression to the later stages is usually fatal (Rosenfeld and Beath, 1964).

Chronic toxicity is characterized by exposure to more moderate levels of selenium (10 to 30 ppm dw) for periods of weeks or months and is often referred to as alkali disease (Rosenfeld and Beath, 1964). Alkali disease occurs when animals ingest plants with protein-bound insoluble selenium (National Research Council, 1980). This disease affects all domestic livestock, but is more prominent in cattle and horses. Signs typically include lack of vitality, anemia, emaciation, stiffness of joints, lameness, rough coat, loss of long hair, hoof sloughing and hoof deformities. Hoof lesions involve a circular break that appears on

the wall of the hoof below the coronary band. Signs of chronic selenosis in swine typically include alopecia, retarded growth, emaciation, lameness, and hoof lesions and deformities, often followed by shedding of hooves (Rosenfeld and Beath, 1964). Raisbeck (2000) speculated that swine are more sensitive to natural sources of selenium than horses, cattle and sheep (least sensitive). Also, the effects of chronic toxicity are suspected to be more prominent in lambs than adult sheep (Gardiner, 1966).

More than 15,000 sheep died of selenium poisoning in a region north of Medicine Bow, Wyoming in the summer of 1907 and 1908 (reviewed by Harr and Muth, 1972; Rosenfeld and Beath, 1964). There have been several reported cases of selenium toxicity resulting from overdosing of selenium supplements (sodium selenite and selenate) in the treatment of nutritional muscular dystrophy (Morrow, 1968; Gabbery and Dickson, 1969; Caravaggi and Clark, 1969; Gabbery, 1970; Hopper et al., 1985; Anderson et al., 1985; Blodgett and Bevill, 1987; Smith et al., 1999). More recent sheep deaths in southeastern Idaho are thought to result from natural selenium exposure in reclaimed mine areas. Approximately 200 sheep deaths in 2001 resulted from drinking lethal amounts of selenium from a spring near a reclaimed phosphate mine (Press, 2001). Recently, more than 300 sheep deaths resulted from grazing a former phosphate mine containing forage with exceptionally high selenium levels (Jones, 2003). Most controlled selenium toxicity studies have been conducted with inorganic selenium (i.e. sodium selenite and selenate). In a study conducted by Glenn et al. (1964a), it was reported that signs of selenium toxicosis in sheep will appear when blood selenium levels reach 2 to 4 ppm selenium. They published another article in which they reported that the minimum toxic level of sodium selenate fed over 100 days was 0.375 mg per pound of body weight per day in sheep (Glenn et al., 1964c).

Grazing studies have showed that sheep require between 0.4 to 1.0 ppm (dw) of selenium in their diet (Puls, 1994). Selenium toxicity may occur when sheep consume plants containing more than 3.0 ppm selenium (Sheep Production Handbook, 1997). Blodgett and Bevill (1987) observed ear drooping and labored respiration following intramuscular injections of sodium selenite at 0.4 to 1.0 mg/kg body weight. As toxicosis progressed, depression was observed, leading to dyspnea, grinding of the teeth, anorexia and mucoid discharges from the nose. Selenium levels in tissues were greatest in the liver, followed by the kidneys, lungs, spleen and myocardium (Glenn et al., 1964b). Similar results were observed by Blodgett and Bevill (1987). They showed that varying doses of sodium selenite resulted in consistently higher concentrations of selenium in the liver than the brain, skeletal muscle and uterus. The development of myocardial degeneration and fibrosis, along with pulmonary congestion and edema was reported by Harr and Muth (1972) in sheep fed 75 mg of selenite per day. The parenteral LD₅₀ for sheep is reported at 0.46 mg Se/kg body weight (Combs and Combs, 1986; Blodgett and Bevill, 1987).

Naturally occurring selenium toxicity is rare in both animals and humans. Studies have shown that some animals may have highly developed senses of taste and smell which enable them to avoid poisonous plants. These types of foraging behaviors have been seen in deer and sheep in new grazing environments (Laycock, 1978; Shamberger, 1986; Burritt and Provenza, 1989). However, animals may be forced to eat toxic selenium plants when grazing conditions are poor because of bad weather, drought or over-grazing (Shamberger, 1986).

Treatments for selenium toxicity are currently being studied. It has been reported that sheep fed high protein diets become more tolerant to selenium intoxication (Rosenfeld and Beath, 1946; Glenn et al., 1964c; Palmer et al., 1980). It was also demonstrated that dietary

sulfate decreased the toxicity of selenate in rats. Methionine was also shown to protect against selenium toxicity in rats, but only when adequate vitamin E levels were present in the diet (National Research Council, 1980). It was discovered that arsenic could counteract the toxicity of seleniferous grains. Also, high protein diets (e.g., linseed meal) have a unique protective activity against chronic selenium toxicosis in chicks and domestic livestock (Rosenfeld and Beath, 1946; Gardiner, 1966; Jensen et al., 1977; National Academy of Science, 1980). The protective mechanism in linseed meal has been linked to cyanohydrin glycosides. Palmer et al., (1980) suggested that this chemical binds to selenium in the tissues in a form that is unavailable for binding to sensitive cellular sulfhydryl sites. This protective mechanism may also occur in balanced mineral mixes that contain sulfur and copper, which have been found to be beneficial in counteracting a high selenium diet and decrease the accumulation of both copper and selenium in the tissues of sheep (Ryssen et al., 1998; Davis et al., 2002). However, Gardiner (1966) reported that cobalt deficiency greatly increases the susceptibility of sheep to selenium toxicity. Preventative measures for protecting grazing livestock continue to be developed. These preventative methods include mapping seleniferous regions, fencing off areas with high selenium in forage and water, avoiding overgrazing on seleniferous vegetation, limiting length of grazing periods on high selenium soils, and adding soil additives to help lower soil pH (Davis et al., 2002).

2.5 Selenium Deficiency

Selenium was not thought to be essential in normal metabolic processes until the late 1950's (Litov et al., 1991). It is now known that selenium deficiency can be a problem for wildlife, domestic livestock and humans. Selenium deficiency in sheep occurs when

selenium levels fall below the normal dose range of less than 0.06 ppm in blood (Oldfield et al., 1963; Horton et al., 1978). However, it has been found that high dietary levels of silver, copper and zinc can induce signs of selenium deficiency (Jensen et al., 1977). The most common selenium deficient areas (< 0.05 ppm selenium in soils) are located in the Pacific Northwest, northern California, the Great Lakes states, the Northeast, the Atlantic coast and Florida (Muth, 1970; National Research Council, 1980; Ullrey, 1992; Tyler et al., 2003). Nutritional muscular dystrophy is a selenium responsive disorder that may be seen in animals consuming diets with insufficient levels of selenium (less than 30 ppb selenium in dry matter). Nutritional muscular dystrophy is characterized by the systemic distribution of degenerative lesions that distinguishes it from local degenerations like fractures or contusions (reviewed by Muth, 1966). Deficiency is mainly seen in young animals (Muth, 1970; Ullrey, 1992). In cases of deficiency, cells have diminished the capacity to detoxify peroxides and both plasma selenium concentrations and glutathione peroxidase activities decrease, resulting in signs of deficiency (Ullrey, 1992). Nutritional muscular dystrophy is typically associated with excessive peroxidation of lipids which results in degeneration, necrosis and subsequent fibrosis of myofibers (National Research Council, 1980; Kaneko, 1986; Smith et al., 1994). Common signs in animals include degenerative diseases of striated muscles (white muscle disease) of calves, lambs, foals and rabbits; hepatitis dietetica (a necrosis of the liver in pigs); and exudative diathesis (subcutaneous edema in birds) (Fishbein 1977; Koller and Exon 1986; Kaneko 1986).

White muscle disease is a disorder that is typically associated with growing animals one to three months old and is considered the most severe form of selenium deficiency in lambs and calves (Koller, 1981). Oldfield et al., (1963) reported that white muscle disease

may occur in nursing ruminants when the maternal diet contains less than 0.02 ppm of selenium and prevented when the dietary selenium concentration was raised to 0.06 ppm selenium. Gross and histopathological signs of white muscle disease in lambs include weakness, difficulty feeding and cardiac degeneration (Oksanen, 1966; Kaneko, 1986; Tinggi, 2003). Other selenium deficiency problems include decreased fertility, abortions and deformations of lambs, decreased wool production, loss of body condition, diarrhea and growth depression in lambs and calves (Koller, 1980; Koller and Exon, 1986; Donoghue and Kronfeld, 1990; Rayman, 2000).

Unthriftiness has been reported to result from a taste aversion of selenium deficient forage (Zuberbuehler et al., 2002). Baker et al., (1989) reported that histopathological lesions such as skeletal muscle degeneration, pancreatic acinar degeneration and neuronal degeneration are similar between selenium toxicity and deficiency cases. The damage caused to the muscles of affected animals may result from calcium salts that are deposited between the muscle fibers in certain parts of the body (Muth, 1970; Wilber, 1980; Hansen et al., 1993). Animals suffering from white muscle disease will have necrosis and mineralization of the heart, with severe lesions located on the right ventricle (Oksanen, 1966; Beytut et al., 2002). When the heart is affected by necrosis and mineralization, sudden heart failure typically results (Hansen et al., 1993). As the muscle degenerates, muscle fibers become dehydrated and less elastic, and waxy in appearance (reviewed by Muth, 1967).

The determination of the selenium status of livestock has been linked with glutathione peroxidase activity. Blood selenium levels have been found to be closely correlated with glutathione peroxidase. Selenium is an essential component of this enzyme, which is incorporated into the red blood cells during erythropoiesis (Ammerman and Miller, 1975;

Koller, 1981). It is thought that glutathione peroxidase neutralizes the effects of hydrogen peroxide and lipid hydroperoxides, which cause cell protein destruction and necrosis (Beytut et al., 2002). Studies have also shown that vitamin E reduces the selenium requirement by maintaining the body selenium in an active form and preventing the destruction of membrane lipids, thereby inhibiting the production of hydroperoxides and reducing the amount of glutathione peroxidase needed to destroy peroxides formed in the cell (McDowell, 1992; Beytut et al., 2002).

Prevention of selenium deficiency includes use of feed from selenium adequate areas or use of selenium supplements. Maintaining the dietary intake of selenium at 0.06 ppm selenium in dry feed will eliminate signs of selenium deficiency in lambs (National Research Council, 1980). Most selenium supplements are in the form of selenites and selenates (National Academy of Science, 1980). The Food and Drug Administration set the maximum level of supplemental selenium at 0.3 ppm per kg of complete feeds for all major food-producing animals (reviewed by Donoghue and Kronfeld, 1990; Ullrey, 1992).

Administration of supplements may be in the form of subcutaneous injections, intraruminal pellets, addition to water and oral supplementation with sodium selenite (Norman et al., 1992). It has been reported that all four methods of supplementation worked effectively for periods ranging from four months to one year after treatment (MacPherson and Chalmers, 1984).

2.6 Absorption, Metabolism and Excretion of Selenium in Domestic Livestock

Selenium absorption is primarily dependent upon its chemical form, the animal species and the presence of other metallic salts with similar electron configurations such as

arsenic (Fishbein, 1980; Combs and Combs, 1986). Absorption of selenium is reported to be significantly lower in ruminants than in monogastric animals. No absorption of selenium occurs in the rumen and abomasum of ruminants. The major site of selenium absorption in ruminants was reported to occur in the small intestine and cecum (National Research Council, 1980). Metabolic breakdown in mammals is reported as rapid and efficient for selenites, selenates and seleniferous compounds such as selenomethionine. This compares to a comparatively low rate and poor absorption of selenide and elemental selenium (Fishbein, 1980). Studies in humans have shown that the absorption of dietary selenium ranges between 55 and 70%. Studies have also suggested that selenium is deposited into tissues at higher concentrations when present in the diet as organic rather than as inorganic selenium (McDowell, 1992). Absorbed selenium is carried in plasma in association with plasma proteins and then is deposited in all tissues. However, when selenomethionine is ingested, animals may quickly accumulate high levels of selenium in tissues (Peterson et al., 1992). Once selenium is in the tissue, it is highly labile (National Research Council, 1980).

The metabolic break down of selenium is affected by chemical forms of selenium, sulfur, arsenic, metals, microorganisms, vitamin E and the amount of previous selenium intake (Figure 2.6.1). Rumen microorganisms may be responsible for the lower absorption rates of selenium in ruminants than those in nonruminants. Dietary selenium is also reduced to insoluble forms by microorganisms. However, these microorganisms are also able to convert inorganic selenium to organic selenium, as well as incorporate organic selenium into bacterial proteins (National Research Council, 1980). Selenium can exert a protective effect against the heavy metals, such as cadmium, lead, mercury and silver. Heavy metals such as lead can reduce selenium absorption (McDowell, 1992).

The major pathways of excretion for selenium are through urine, feces (highest levels excreted by ruminants) and exhalation. Minor pathways include the mammary glands and placenta (Glenn et al., 1964a). Factors that affect the amount of selenium elimination are dependent upon the level of intake, the form administered, composition of the diet and other variables such as arsenic exposure (Combs and Combs, 1986). Urine is the major pathway of excretion in monogastric species. Most selenium in feces is from selenium that has not been absorbed from the diet. The feces also include small amounts of selenium excreted from biliary, pancreatic and intestinal secretions (National Academy of Science, 1980). Selenium excretion in ruminants is dependent on the method of administration. Ingested selenium typically is excreted with feces but injected selenium is mainly excreted in urine. Pulmonary excretion is considered the major route of selenium excretion only when toxic concentrations have been consumed (Ammerman and Miller, 1975; McDowell, 1992).

2.7 Selenium Toxicity: Biochemical Functions and Mode of Action

Historically, selenium has been associated with vitamin E and antioxidant activity. However, selenium was recognized in 1950's as having nutritional significance and possibly being an essential nutrient (Combs and Combs, 1986). Selenium was only known for its toxicity before this period. Currently, selenium toxicity still occurs in domestic livestock and wildlife. Research is ongoing to understand the mechanism of toxicity and mode of action of selenium.

Selenium closely resembles sulfur in chemical properties such as atomic size, bond energies, ionization potentials and electron affinities. The major difference between these

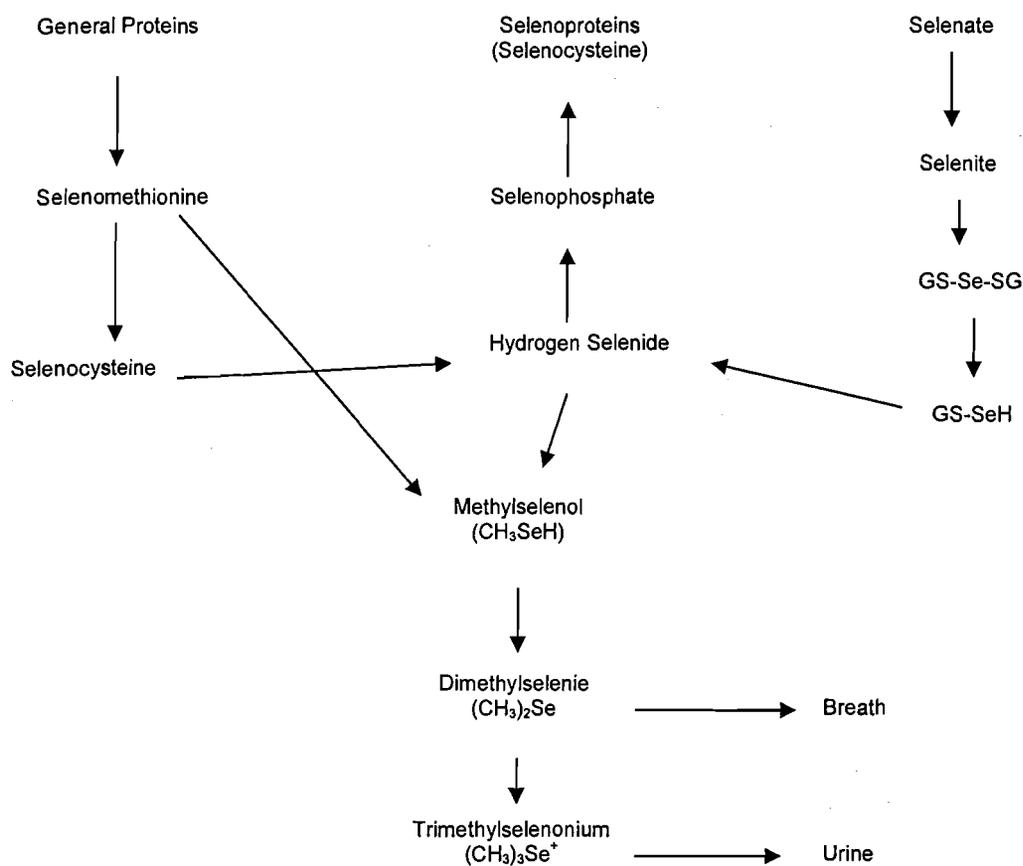


Figure 2.6.1 *Metabolism of Selenium in Animals. Adapted from Tinggi, 2003.*

two elements is that selenium exists as a reduced quadrivalent form and sulfur occurs as an oxidized quadrivalent. Other differences are seen in acid strengths. Selenium hydride (H₂Se) is reported as a stronger acid (pK_a = 3.7) than sulfur hydride (H₂S, pK_a = 6.9) (Harr and Muth, 1972; Spallholz, 1994; Tinggi, 2003). Due to similar chemical properties selenium can readily substitute for sulfur in many enzymes with disulfide bridges. It has been hypothesized that when selenium substitutes for sulfur in keratin the result is a weakened protein structure that may result in hoof and hair loss, and hoof deformations (Raisbeck, 2000).

Experiments in animals have shown that selenium toxicity is accompanied by indicators of oxidative injury. However, the mode of action of selenium at both the cellular and molecular level is not yet fully understood. It has been suggested that selenium toxicity may be due to the interaction of selenite with glutathione to form reactive selenotrisulfides to produce toxic superoxide and hydrogen peroxide (Spallholz, 1994; Spallholz, 1997; Tinggi, 2003).

The main functions of selenium are exerted by selenoproteins. Selenoproteins contain one or more atoms of selenium. The most common chemical form of selenium in animals is selenocysteine (Figure 2.6.1). Selenocysteine is made up of the same carbon skeleton as cysteine, the only difference being that selenium replaces sulfur. It has been reported that selenocysteine, rather than cysteine, in an enzyme active site increases enzyme activity (Burk, 2002).

Glutathione peroxidase (GSH-Px) was first identified as a selenoenzyme in the early 1970's. Functions of GSH-Px include the protection of cellular membranes and lipid containing organelles from peroxidative damage by inhibition and destruction of endogenous peroxides (Behne et al., 2001; Koller et al., 1986). The removal of hydrogen peroxide and lipid hydroperoxides by reducing glutathione, eliminates toxic molecules and signals the presence of toxic molecules to the cell (Burk, 2002) (Figure 2.7.1). Other functions include protecting cellular and subcellular membranes from oxidative damage (McDowell, 1992).

Additional biochemical functions of selenium, unrelated to that of GSHP-Px, include a specific selenoprotein of spermatozoa that serves as a mitochondrial structural protein or as an enzyme. Selenium also plays a role in RNA through the incorporation of selenium into

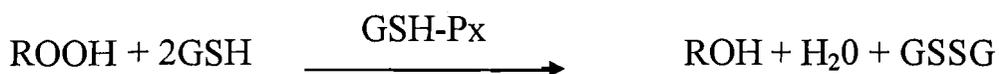


Figure 2.7.1 Reduction of H_2O_2 (lipid hydroperoxides or sterol hydroperoxides) by glutathione peroxidase and equivalents of reduced glutathione (GSH). Adapted from Shamberger, 1986.

purine or pyrimidine bases. One role of selenium in RNA function is reported to be the incorporation of selenium into the stop codon UGA (made up of uracil, guanine and adenine) as a codon for selenocysteine, which results in the termination of translation (Bermano et al., 1996). Studies have also suggested a role of selenium in prostaglandin synthesis and essential fatty acid metabolism. Lastly, selenium and vitamin E are needed for normal immune responses by livestock (McDowell, 1992). Selenium is currently being studied as an anticarcinogen. Natural products formed from selenium in plants can give rise to more active chemopreventive metabolites in animals, compared to chemopreventive products formed in animals from inorganic selenium (Clark et al., 1996; Ganther, 1997).

2.8 Experimental Objectives/Rationale

The objective of this research was to collect baseline data from sheep grazing on reclaimed mining sites with elevated levels of selenium in the forage, water and soil. The baseline data monitoring included selenium levels in blood, serum, feces, urine and selected tissues and health status monitoring of complete blood counts and a panel of serochemistries which may be related to selenium toxicity. It was anticipated that these data would lead to a better understanding of the parameters and predictors involved in the death of sheep in these areas that would assist in lessening future problems.

3.0 Materials and Methods

3.1 Experimental Design

Seventy-two Columbia ewes and their Columbia-Suffolk lambs were used in the study. The sheep consisted of 36 adult ewes (2-5 years of age) and their spring lambs (<1 year of age). The sheep were obtained from a local sheep rancher before the initiation of the study. These sheep were apart of a larger range flock that typically grazes reclaimed mining areas for part of the year. A typical grazing cycle begins late May and continues through early August. A local veterinarian performed a basic physical examination (heart, air passages, body temperature, eyes, ears, mouth, skin/coat, abdomen and general body condition) of all sheep prior to the initiation of the study. Sheep in good physical conditioning and health status were used in the experiment. Sheep with obvious health ailments were not included in the study.

Sheep were divided into three groups of 24. The groups consisted of 12 adult ewes and their corresponding lambs. All sheep were held on a nearby ranch and given access to feed and water containing normal background levels of selenium for the first two sampling periods of the study (baseline phase – days 0-21). The animals were then moved to pens on mine sites for the next four weeks (exposure phase – days 21-49). Two groups were held on reclaimed mine sites that had elevated selenium in the soil and forage. One of the groups (high selenium - HiSe) received drinking water from a source on the mine site with elevated selenium levels (340 - 420 ppb). The other group (low selenium - LoSe) received drinking water from a source with normal background selenium levels (< 50 ppb). The control group (Con) was held in a nearby area of similar terrain containing forage and water with normal background selenium levels. Drinking water was pumped by a Craftsman 1/12 horse-

powered portable utility pump that was powered by a 5 horse-power engine (Coleman Generator Ultra), from either the selenium contaminated or control sources. The water was pumped through a standard garden hose into 30 gal polyethylene barrels and transported by trucks to the different sites every other day. The water was then poured into 50 gal Rubbermaid stock tanks located at the study sites. Water and natural forage were available *ad libitum*. Following the exposure phase of the study, all animals were transported by truck back to the baseline site and held for two weeks on forage and water with normal background selenium levels (depuration phase). Two additional blood collections were conducted following the depuration phase (supplemental phase).

The sheep on reclamation mine sites were held in pens surrounded by three-wire, wind-up reel, portable electric fences. The fence was supported by light duty fiberglass posts and powdered by Solar-Pak Magnum fence chargers. Pen sizes (approximately 50 x 100 yards) were determined by manageable terrain and estimated feed consumption rate (average dry matter intake for 154 lb ewe is 1.7% for maintenance; Lyons at el., 1995; Umberger, 2001). Each pen contained approximately eight steel gate panels (4 feet high and 10 feet long). Two to three panels for each pen contained plywood sheets attached to create a solid panel. These solid panels were also used to build weather shelters by placing the solid panels above the regular panels, providing minor protection from wind, rain and sun. The corrals (panels) were used to assist with handling individual sheep during sample collection and health examinations. Sheep were herded into the corrals by sheep dogs and handlers. Once the sheep were inside the corral, they were pushed into a narrow corner of the corral that forced the sheep into a single line formation (head to tail). This part of the corral was

constructed by converging two panels into each other forming a V-like fence structure or chute.

The sheep were monitored daily by an on-site research assistant who provided written assessments on general field and animal health monitoring activities. This included forage growth within a pen, grazing activity (plant palatability and preferences), weather monitoring, fence stability and any other problems encountered. Blood, serum, feces and urine samples were collected weekly (see below for details) from sheep in each group by a local veterinarian. Blood samples were shipped on ice to the University of Idaho Analytical Sciences Laboratory (UIASL). Serum was collected from centrifuged whole blood in 7 ml no additive vacutainer tubes and sent to Gritman Medical Center in Moscow, ID for complete blood counts and comprehensive serochemistry profiles. A duplicate set of blood/serum samples were sent to the UIASL for selenium.

3.2 Blood Analysis

Blood collections were performed weekly by the local veterinarian and the onsite assistant. Blood was collected by venopuncture into the jugular vein of the sheep using a 16 gauge x 1 ½ inch needle and 35 cc luer-lok disposable syringe by (Monoject – Fisher Scientific). Blood samples, as whole blood, plasma or serum were analyzed for selenium, clinical chemistries and pregnancy (Con and HiSe). Clinical chemistries included: urea, creatinine, total bilirubin, total protein, albumin, globulin, calcium, potassium, sodium, alkaline phosphatase, alanine transferase, aspartate aminotransferase and total carbon dioxide. Complete blood counts included: white blood cell count, red blood cell count, hemoglobin, hematocrit, and platelet counts.

Hematology and clinical chemistry analyses were performed by Gritman Medical Center Laboratory, a Joint Commission Accreditation Health Organization (JCAHO) certified facility. An automated complete cell count (CBC) including white blood cells, red blood cells, hemoglobin, hematocrit and WBC differential was analyzed on the CellDyn 3000 (Abbott Diagnostics) according to the manufacturer's parameters. If automated criteria were not met, a manual differential was performed. The clinical chemistries were analyzed on a Dade Dimension RXL (Dade Behring) according to manufacturer's parameters. Required quality control and quality assurance were performed for all procedures.

Blood and serum was analyzed for total selenium by UIASL using hydride generation by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Perkin-Elmer P-40). Initial blood samples were digested by heating in nitric acid and then boiled in sulfuric and perchloric acids. This step converts selenium species into selenate, which is then reduced to selenite. Selenite is then reduced by acidic sodium borohydride to selenic hydride that is then measured at 196 nm (Tracy and Moller, 1990).

Three weeks after beginning the selenium exposure, rams were placed in the Con and HiSe pens for breeding with the ewes. Pregnancy tests were performed using blood samples from the Con and HiSe ewes after the depuration phase. Pregnancy tests were done by radioimmunoassay with the exact method by Willard et al. (1994).

3.3 Fecal and Urine Analysis

Fecal and urine samples were collected weekly from two selected ewes in each group to generally assess selenium elimination. Fecal samples were collected by rectal palpation to prevent contamination. Each sample was stored in a chilled cooler and shipped in labeled

Ziploc bags. Urine samples were collected by blocking the ewe's nose which momentarily stops respiration and forces urination. Urine was collected in 225 ml conical Falcon centrifuge tubes. Each sample was sealed with parafilm, chilled and shipped with fecal samples to UIASL. The method for total selenium analysis for these biological tissues is the same as for blood (Tracy and Moller, 1990).

3.4 Water Analysis

Samples of water were taken directly from the drinking troughs at each site weekly for selenium analysis. At each site, one 125 ml filtered and unpreserved and one 250 ml unfiltered and preserved sample was taken. The filtering processes was performed by using a 60 cc Luer-Lok disposable brand syringe (Fisher Scientific) to collect water and then filtering through a 25 mm diameter (0.22 μm pore size) syringe filter (Fisher Scientific). For the unfiltered sample, a 500 ml plastic beaker was used to pour the water sample into the 250 ml collection bottle. Samples were chilled and shipped to UIASL. Analysis was performed by ICP-AES (Tracy and Moller, 1990).

3.5 Plant Analysis

Randomized, replicate plant samples of the dominant forage plant varieties were collected and analyzed weekly from each pen. A randomized circular plot (less than 1 meter diameter) method was used to collect both plant and soil samples. Plant samples included all surface vegetation within the circular plot. The plant specimens were identified and separated into labeled Ziploc bags, chilled and shipped to UIASL for analysis. Plant species and stage of growth was identified with the support of the University of Idaho Herbarium

botanists and onsite assistance from United States Forest Service. Total selenium analysis was analyzed by the ICP-AES (Tracy and Moller, 1990).

3.6 Soil Analysis

Randomized soil samples from each pen were taken weekly and analyzed for selenium. Soil samples were taken at a depth profile of 0 to 25 cm and 25 cm to 50 cm using standard 5 cm soil coring tool. Soil samples were shipped to the UIASL for analysis of selenium. Total selenium analysis was analyzed by the ICP-AES (Tracy and Moller, 1990).

3.7 Necropsies

Necropsies were conducted by a local veterinarian on the sheep that died during the study and also on one ewe and lamb from each group (Con and HiSe) that were terminated after the exposure phase. The sheep were examined for gross lesions (liver, kidney, skeletal muscle, heart, spleen and lung). Tissue samples were collected for histopathological examination by a veterinary pathologist at the Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, Washington. Additional liver, kidney, skeletal muscle, heart, spleen and lung, were collected and selenium analysis was performed by the UIASL and results reviewed by a veterinary toxicologist. Selenium analysis in the tissues was performed by the method of Tracy and Moller (1990).

3.8 Statistical Analysis

Blood, serum, serochemistries, urine and feces were analyzed by the SAS for Windows (1999) in consultation with the College of Agriculture and Life Sciences Statistical

Programs. Each sample was analyzed for basic statistical measures (e.g., mean, median, mode, standard deviation, variation, range and interquartile range), with a normality test performed for each week. A least-squares analysis of variance using the General Linear Model (GLM) procedure of SAS was used to compare the treatment means of dependent variables prior to exposure (baseline). The statistical model included comparisons of treatment groups, age and the interaction between age and treatment group. For each week of exposure or depuration, analysis of covariance was used to compare treatments using the baseline values of each animal as covariances. These models included treatment group, age, the interaction between the treatment group and age and also the baseline values.

4.0 Results

4.1 Total Selenium in Blood and Serum

Whole blood selenium levels increased steadily during the exposure phase of the study (days 22-49) in both the HiSe lambs and ewes (Figure 4.1.1). The mean peak selenium concentrations on day 49 were 1.36 ppm in the lambs and 1.27 ppm in the ewes compared to mean levels of 0.32 ppm in the lambs and 0.34 ppm in the ewes in the baseline of the same group. The whole blood selenium concentrations in the HiSe group decreased initially at the beginning of the depuration phase (days 49-56) and then plateaued until day 70 when they again began to decrease slowly to levels of 1.17 ppm in the lambs and 0.98 ppm in the ewes on day 93. Whole blood selenium levels were higher ($p < 0.05$) in both the HiSe lambs and ewes when compared to the Con during the entire exposure phase and the depuration phase (Table 4.1.1). Whole blood concentrations of selenium in the HiSe animals tended to be greater ($p < 0.05$) in the lambs compared to the ewes during most of the exposure and depuration phases but the exposure curves followed the same general pattern. The Con group maintained blood selenium levels in the range of 0.05-0.14 ppm for the entire study.

Whole blood selenium levels also increased steadily in the LoSe lambs and ewes during the exposure phase of the study. Data was only collected from this group through day 42 when they disappeared from the study site. Whole blood selenium levels in this group were lower ($p < 0.05$) than the HiSe group and higher ($p < 0.05$) than the Con group. In this group, whole blood selenium was not different between ewes and lambs during the sampling period.

Serum selenium levels in the HiSe animals increased steadily during the exposure phase to peak at 0.94 ppm in the lambs and 1.04 ppm in the ewes on day 49 (Figure 4.1.2).

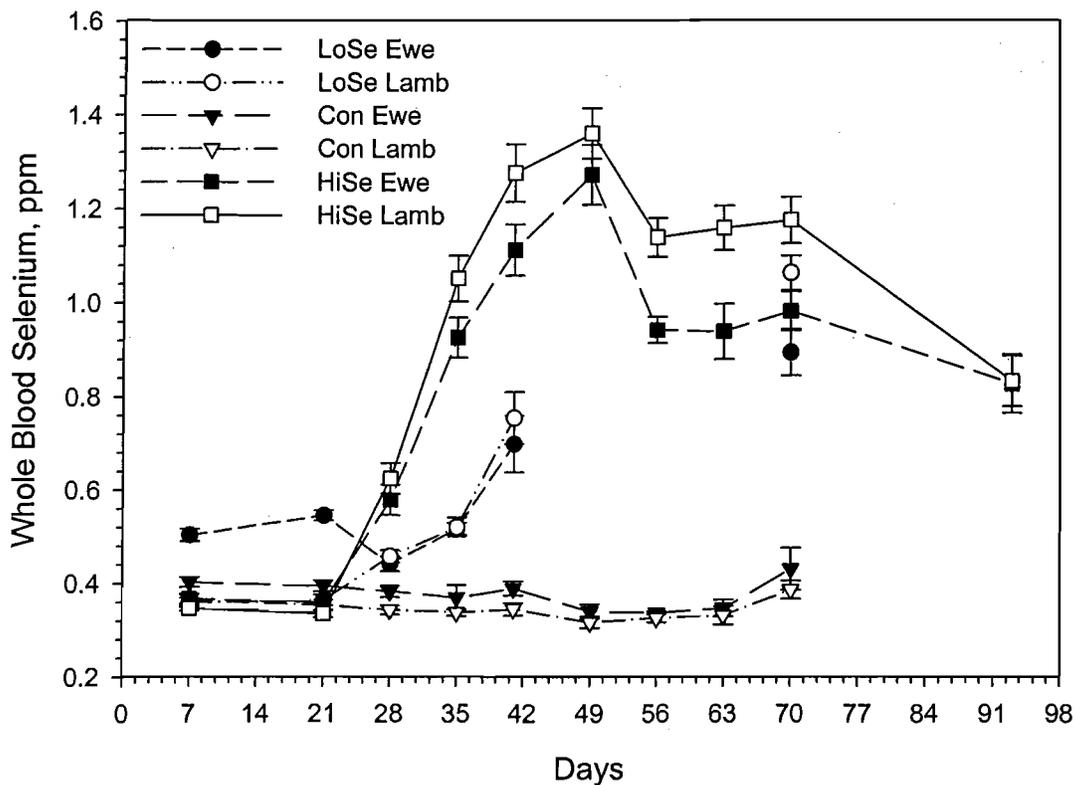


Figure 4.1.1 Total whole blood selenium in sheep (mean \pm standard error).
Low Selenium (LoSe) – Control (Con) – High Selenium (HiSe)

Table 4.1.1 Level of significance (<0.05) of whole blood selenium in sheep.
Control Ewe (CE) – Control Lamb (CL) – High Ewe (HE)
High Lamb (HL) – Low Ewe (LE) – Low Lamb (LL)

Days	Level of Significance < 0.05
28	CE – HE; CE – LE; CL – LL; CL – HL; HE – LE; HL – LL
35	CE – HE; CE – LE; CL – HL; CL – LL; HE – HL; HE – LE; HL – LL
41	CE – HE; CE – LE; CL – HL; CL – LL; HE – HL; HE – LE; HL – LL
49	CE – HE; HE – HL
56	CE – HE; CL – HL; CL – LL; HE – HL
63	CE – HE; CL – HL; HE – HL
70	CE – HE; CL – HL; CL – LL; HE – HL; LE – LL; HL – LL

The serum selenium levels decreased rapidly to near the Con group and baseline levels during the depuration phase. Serum selenium levels in the LoSe animals also increased

steadily during the exposure phase to peak at 0.33 ppm in the ewes and 0.31 ppm in the lambs on day 35. This increase in serum selenium concentrations in the LoSe groups was greater ($p < 0.05$) than the Con group but less ($p < 0.05$) than the HiSe group (Table 4.1.2). There was no difference in accumulation of selenium in sera of lambs and ewes in either of selenium exposure groups. The Con group maintained serum selenium levels ranging from 0.06 to 0.13 ppm throughout the study.

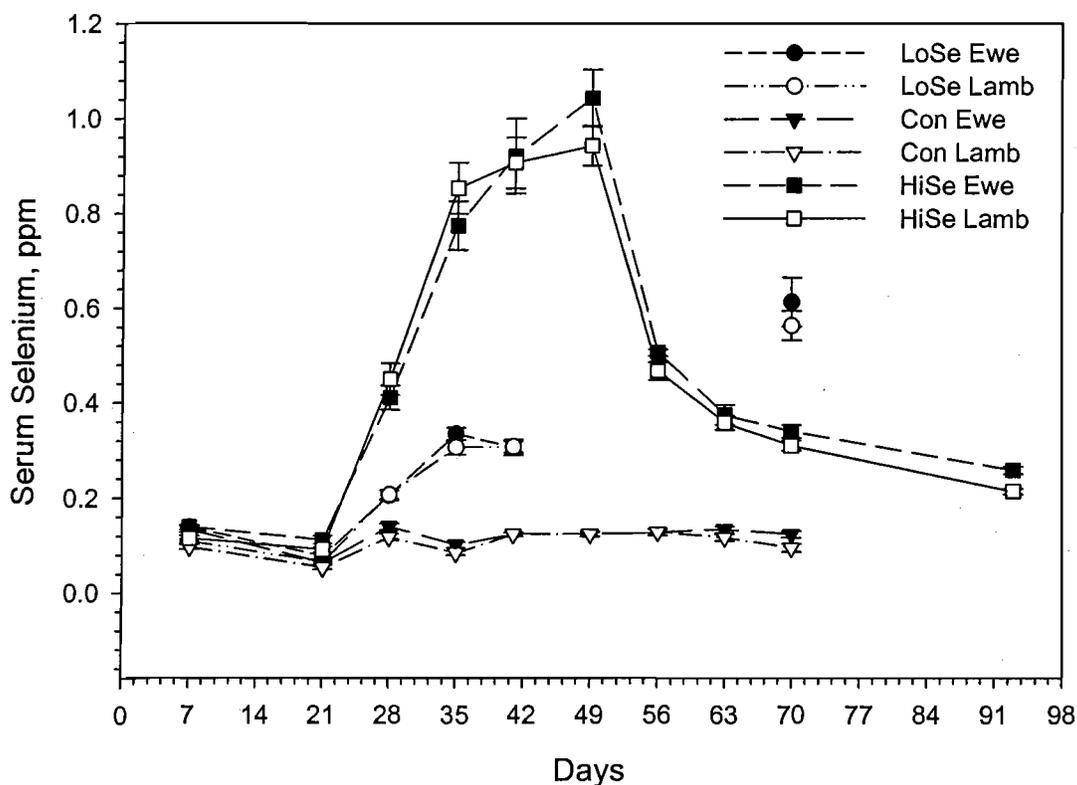


Figure 4.1.2 Total blood serum selenium in sheep (mean \pm standard error).
Low Selenium (LoSe) – Control (Con) – High Selenium (HiSe)

4.2 Clinical Chemistry

Chloride, potassium, sodium, eosinophils, hemoglobin, monocyte, platelet, red blood cells, white blood cells, creatinine, urea, total protein, alkaline phosphatase, alanine

Table 4.1.2 *Level of significance (< 0.05) of blood serum selenium in sheep.*
Control Ewe (CE) – Control Lamb (CL) – High Ewe (HE) –
High Lamb (HL) – Low Ewe (LE) – Low Lamb (LL)

Days	Level of Significance < 0.05
21	CE – HE; CL – HL; HE – LE; HL – LL
28	CE – HE; CE – LE; CL – HL; CL – LL; HE – LE; HL – LL
35	CE – HE; CE – LE; CL – HL; CL – LL; HE – LE; HL – LL
41	CE – HE; CE – LE; CL – HL; CL – LL; HE – LE; HL – LL
49	CE – HE; CL – HL
56	CE – HE; CL – HL
63	CE – HE; CL – HL
70	CE – HE; CE – LE; CL – HL; CL – LL; HE – LE; HL – LL

aminotransferase and total bilirubin numbers were all within the normal range for sheep throughout the study (Figures 7.1.1 – 7.1.15). However, there were consistent differences observed in these parameters between either the selenium exposed animals and the Con group or between the ewes and their respective lambs (Figure 7.1.4 and Figures 7.1.9 – 7.1.15). There were observed differences in the eosinophil counts between the Con lambs and ewes on days 21-49 (Figure 7.1.4). White blood cell numbers were occasionally below the normal range (4 – 12 1000/mm) in all groups on days 21-63 (Figure 7.1.9). Creatinine concentrations were different ($p < 0.05$) in the Con ewes compared to the HiSe ewes on days 21-63 and the LoSe ewes on days 35-42 (Figure 7.1.10 and Table 7.1.10). The Con lambs were higher ($p < 0.05$) than the HiSe lambs in mean creatinine concentrations on days 35-56. Mean urea concentrations in the HiSe ewes and lambs were higher ($p < 0.05$) than the Con ewes and lambs on days 28-49 (Figure 7.1.11 and Table 7.1.11). The mean blood urea concentrations in the HiSe lambs were higher ($p < 0.05$) than the HiSe ewes on days 35-49. All groups of ewes had higher total blood protein concentrations than their lambs on days 21-49 (Figure 7.1.12 and Table 7.1.12). Total blood protein concentrations in serum of ewes in the HiSe were greater ($p < 0.05$) than the HiSe lambs on days 21-63. Alkaline phosphatase

mean concentrations in all groups of lambs were higher than the ewes on days 21-63 (Figure 7.1.13 and Table 7.1.13). The HiSe lambs had higher ($p<0.05$) alkaline phosphatase levels than the HiSe ewes on days 21-63. Alanine aminotransferase mean concentrations in the Con lambs were higher ($p<0.05$) than the HiSe lambs on days 28-63 (Figure 7.1.14 and Table 7.1.14). The Con ewes had significantly higher ($p<0.05$) alanine aminotransferase concentrations than the HiSe ewes on days 28-49. The LoSe ewes had higher ($p<0.05$) levels of alanine aminotransferase than the LoSe lambs on days 21-42. Total bilirubin concentrations in the Con lambs were higher ($p<0.05$) than the HiSe lambs on days 21-49 (Figure 7.1.15 and Table 7.1.15). The Con ewes were higher ($p<0.05$) in total bilirubin concentrations than the HiSe ewes on days 49-63.

Calcium, hematocrit, globulin and aspartate aminotransferase were consistently above the normal ranges for sheep (Figures 7.2.1 – 7.2.4). However, hematocrit, globulin and aspartate aminotransferase were the only parameters for which consistent differences were observed between selenium exposed animals (Figure 7.2.2 – 7.2.4). Mean hematocrit levels were elevated above the normal range (24 - 50%) in the Con ewes on days 21-49 (Figure 7.2.2 and Table 7.2.2). The Con ewes had higher ($p<0.05$) hematocrit levels than the HiSe ewes on days 21-42. Mean globulin concentrations were elevated (3.4 – 4.8 gm/dl) in all sheep on days 21-63 (Figure 7.2.3 and Table 7.2.3). Mean globulin concentrations in the HiSe ewes were significantly greater ($p<0.05$) than the HiSe lambs on days 21-63. All ewes had higher globulin concentrations than their respective lambs on days 21-56. Aspartate aminotransferase concentrations in all sheep decreased steadily from days 21-63 (Figure 7.2.4 and Table 7.2.4). Aspartate aminotransferase concentrations in all sheep were elevated above normal range (81 – 143 U/L) on days 21-28. However, the Con ewes were higher

($p < 0.05$) in aspartate aminotransferase concentrations than the Con lambs on days 21-49.

Aspartate aminotransferase concentrations were elevated above the normal range in the Con ewes on days 21-49.

Albumin and total carbon dioxide were consistently below the normal ranges for sheep (Figures 7.3.1 – 7.3.2). No consistent significant differences were found between the selenium exposed animals and the Con group or between the ewes and their respective lambs with respect to these parameters.

4.3 Pregnancy Report

Pregnancy testing of the HiSe group showed that one ewe (1 out of 9) was not pregnant. Two ewes from the Con group were not pregnant (2 out of 11).

4.4 Necropsy and Histopathology

Skeletal muscle tissue samples from the HiSe sheep necropsied after the exposure phase had selenium levels (ppm wet weight) of 0.71 ppm in the ewe and 1.40 ppm in the lamb after the exposure phase (Table 4.4.1 and 4.4.2). The Con ewe had skeletal muscle tissue selenium levels of 0.12 ppm. Selenium concentrations in the kidneys of the ewes and lambs in the HiSe group were 3.00-4.00 ppm following the exposure phase. The Con lamb and ewe had kidney selenium concentrations of 0.72 – 1.40 ppm respectively. Liver selenium concentrations ranged from 5.00-6.00 ppm in the HiSe ewe and lamb. Liver selenium concentrations in the Con lamb and ewe were 0.41-0.46 ppm. All necropsied sheep had evidence of sarcocytosis either in the heart and skeletal muscle. Myocarditis was associated with myocardial degeneration in the ewes from the Con and HiSe groups.

Eosinophilic enteritis was noted in all sheep. Cestodes parasites (tapeworms) were found in the Con lamb. Mild pulmonary edema was noted in the Con ewe and lamb. Neuron and axon degeneration was noted within the brainstem of the Con ewe.

One ewe in the HiSe pen died 14 days after beginning selenium exposure (Table 4.4.2). Selenium tissue concentrations were 0.70 ppm in the skeletal muscle, 1.90 ppm in the kidney and 3.90 ppm in the liver. Myocardial degeneration and pulmonary edema were observed in this ewe. Cytosegrosomes within hepatocytes were also noted in liver sections. Mild subclinical eosinophilic enteritis was present, which was associated with mild intestinal parasitism. Aspirated ingesta within the lung was noted but no evidence of inflammatory response to foreign materials was identified.

Table 4.4.1 *Diagnostic reference ranges of selenium (ppm) in sheep tissues. Adapted from the Veterinary Clinical Pathology Laboratory, Washington State University (1998).*

Tissues	Approximate Reference Ranges		
	Adequate	High	Toxic
Liver	0.25-1.50	2.00-10.00	> 15.00
Kidney	0.90-3.00	4.00-6.00	> 6.00
Skeletal Muscle	0.09-0.40	0.40-0.60	> 0.60
Heart	0.25-0.40		
Spleen	0.30-0.90		
Lung	0.20-0.30		

4.5 Total Selenium in Urine and Feces

Urine selenium levels increased rapidly on days 28-35 in the HiSe group (Figure 4.5.1). The mean peak selenium concentration on day 35 in the HiSe group was 7.4 ppm. The urine selenium concentrations in the HiSe group decreased rapidly on days 35-41. These levels then gradually decreased after day 41 and reached Con group levels on day 63. Urine selenium concentrations were below detectable levels in all groups on day 21. The Con group maintained urine selenium concentrations at about 0.1 ppm on days 28-63. Urine

Table 4.4.2 *Selenium tissue levels (ppm) in the sheep that died during exposure and sheep that were necropsied after the exposure phase.*

Tissues	HiSe		Con		HiSe	
	Dead	Ewe	Ewe	Lamb	Ewe	Lamb
Liver	3.90 ^b		0.46	0.41 ^a	5.00 ^b	6.00 ^b
Kidney	1.90 ^a		1.40	0.72 ^a	3.00 ^a	4.00 ^b
Skeletal Muscle	0.70 ^c		0.12	n/a	0.71 ^c	1.40 ^c
Heart	n/a		0.26	0.28 ^a	1.50	2.40
Spleen	1.40		0.37	0.32 ^a	2.00	2.40
Lung	n/a		n/a	n/a	n/a	n/a

Control (Con) – High Selenium (HiSe) – Not Available (n/a)

a = adequate b = high c = toxic

selenium concentrations in the HiSe group were higher ($p < 0.05$) than the Con group on days 28-56 and the LoSe group on days 28-42. The LoSe group had urine selenium levels that were higher ($p < 0.05$) than the Con group on days 28-41.

Fecal selenium levels increased steadily in the HiSe group on days 21-42 (Figure 4.5.2). The peak selenium concentration in the HiSe animals on day 42 was 30.0 ppm (dry weight). Fecal selenium levels in the HiSe animals decreased gradually between days 42-49 then rapidly on days 49-56. These selenium levels reached Con group levels on day 56. Fecal selenium levels in the LoSe animals peaked on day 35 at 10.2 ppm. The Con group maintained fecal selenium concentrations at 0.1 ppm through day 63. Fecal selenium concentrations in the HiSe animals were higher ($p < 0.05$) than the Con animals on days 28-49 and the LoSe animals on days 28-42. The LoSe group was higher ($p < 0.05$) in fecal selenium levels than the Con group on days 28-42.

4.6 Total Selenium in Water

Water selenium levels during the exposure phase provided to the Con and LoSe groups ranged from below detectable levels to 1.65 ppb (Table 4.6.1). The HiSe group water

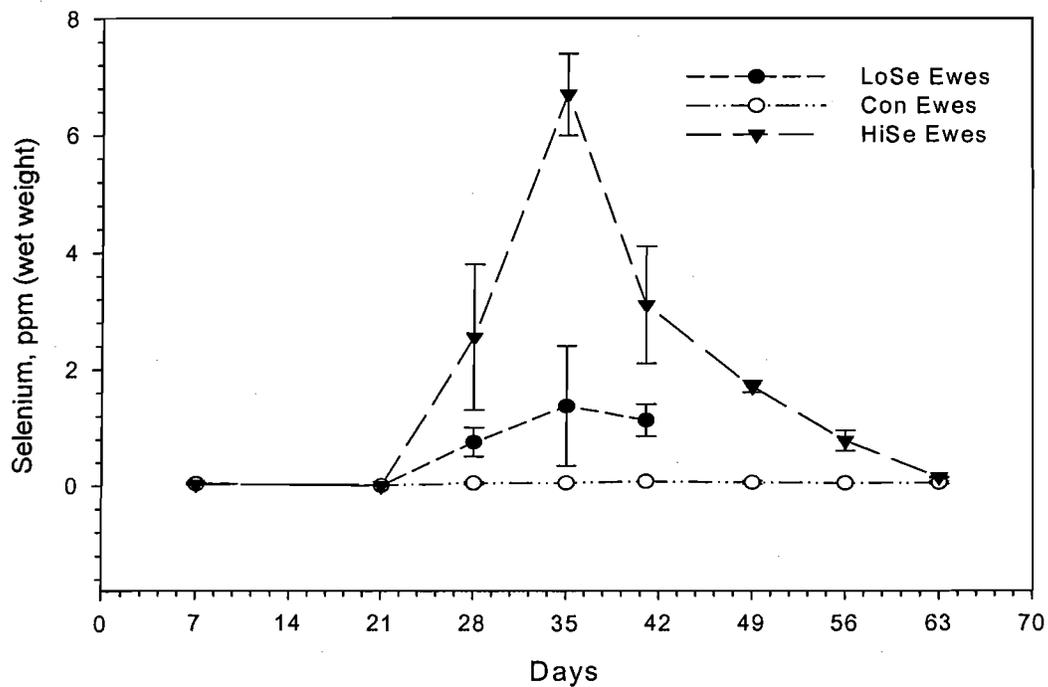


Figure 4.5.1 Total selenium levels in sheep urine (mean \pm standard error).
Low Selenium (LoSe) – Control (Con) – High Selenium (HiSe)

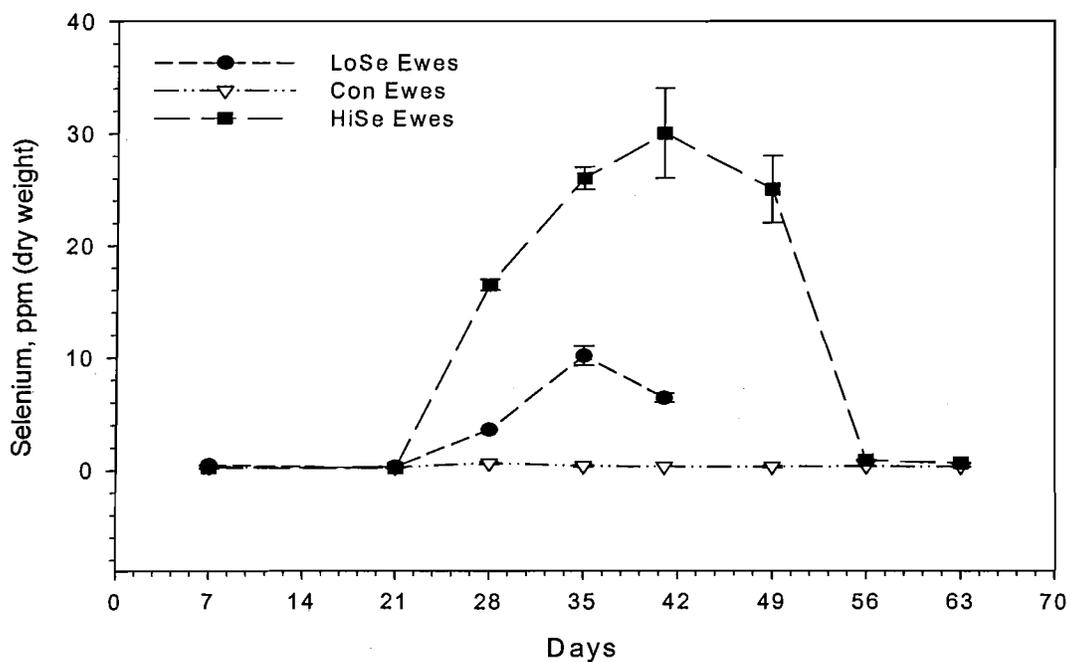


Figure 4.5.2 Total selenium levels in sheep feces (mean \pm standard error).
Low Selenium (LoSe) – Control (Con) – High Selenium (HiSe)

selenium levels ranged from 340 to 415 ppb during the exposure phase with a mean of 375 ppb.

Table 4.6.1 Mean selenium concentrations (ppb) in drinking water.

Group	Weeks				
	2	3	4	5	6
Con/LoSe	1.65	BDL	1.3	n/a	BDL
HiSe	350	340	415	n/a	395

Control (Con) – Low Selenium (LoSe) – High Selenium (HiSe)
Below Detectable Levels (BDL) – Not Available (n/a)

4.7 Total Selenium in Plants

Plants selenium levels collected from the Con pen had concentrations within the marginal range (Table 4.7.1). Selenium concentrations ranged from 0.04 ppm (dw) in the intermediate wheatgrass to 0.20 ppm in the smooth brome grass (Table 4.7.2). Mean plant selenium levels were 0.15 ppm in the Con pen. Plants in the LoSe pen had selenium concentrations that ranged from 0.05 ppm in the smooth brome grass to 13.0 ppm in the alfalfa. The plants in the HiSe pen had selenium levels that ranged from 0.33 ppm in the alfalfa to 110.0 ppm in an unidentified plant species.

Table 4.7.1. Selenium reference levels in a sheep diet (ppm dry weight).
Adapted from Puls, 1994.

	Diet (ppm)
Deficient	0.02 - 0.10
Marginal	0.10 – 0.25
Adequate	0.40 – 1.00
High	3.00 – 5.00
Toxic	5.00 – 25.0

Table 4.7.2 Mean and range selenium values (ppm) in major plant species.

Plant Se Levels in Major Plant Species			
Groups	Species	Range	Mean
Con	Intermediate Wheatgrass	0.042 - 0.170	0.102 ^b
	Smooth Bromegrass	0.096 - 0.200	0.172 ^b
	Alfalfa	2.600 - 13.000	7.960 ^e
LoSe	Intermediate Wheatgrass	0.480 - 3.200	1.224 ^c
	Smooth Bromegrass	0.046 - 3.000	1.031 ^c
	Unidentified Grass Species	0.130 - 0.400	0.265 ^b
	Alfalfa	0.330 - 49.000	15.761 ^e
HiSe	Intermediate Wheatgrass	0.570 - 14.000	7.285 ^e
	Smooth Bromegrass	0.530 - 14.000	7.155 ^e
	Unidentified Grass Species	9.400 - 110.000	59.700 ^e
	Alfalfa	0.330 - 49.000	15.761 ^e

a = deficient b = marginal c = adequate d = high e = toxic

4.8 Total Selenium in Soil

Soil selenium levels ranged from 0.62-2.10 ppm (dw) in the Con pen (Table 4.8.1).

The mean soil selenium concentration for the Con pen was 0.92 ppm. Selenium in the soil of LoSe pen ranged from 14.0-59.0 ppm with a mean value of 9.92 ppm. Selenium levels in the soil of the HiSe pen ranged from 3.9-90.0 ppm with a mean value of 35.48 ppm.

Table 4.8.1 Mean and range selenium levels in soil (ppm dry weight).
Control (Con) – Low Selenium (LoSe) – High Selenium (HiSe)

Group	Range	Mean
Con	0.62 – 2.10	0.92
LoSe	14.00 – 59.00	29.92
HiSe	3.90 – 90.00	35.48

4.9 Grazing Observations

Sheep in the HiSe pen had an early aversion to all forage and water until day 38. All animals in this group grazed a variety of forage including alfalfa, smooth brome grass and intermediate wheatgrass, with the main consumption consisting of the leaves. Sheep in the LoSe pen were selective foragers. These sheep preferred alfalfa, followed by smooth brome grass and lastly the intermediate wheatgrass. The LoSe group consumed all of the alfalfa plant and smooth brome grass. The intermediate wheatgrass was consumed in lesser amounts by these sheep and it was observed that only the leaves were consumed, leaving the stem and roots. The Con had no preference for any particular grass species and consumed all forage within their pen with equal preference.

5.0 Discussion

Reclaimed phosphate mining sites in southeastern Idaho have elevated levels of selenium in the soil, water and plants. In 2001, the death of approximately 200 sheep grazing in this area were attributed to selenium toxicity (Montgomery Watson, 1999). There have been other isolated incidences of sheep deaths in this area for which the cause has not been fully understood. Some of these sheep deaths appear to be sporadic events with no consistent etiology or chronology. One goal of this study was to better understand the dynamics of grazing sheep in these reclaimed mining areas under semi-controlled conditions. The death of one of 24 sheep in the HiSe group was attributed to selenium toxicity. Histopathological lesions in the heart and lungs, and elevated levels of selenium in the tissues were observed in the dead ewe were consistent with selenium toxicity. The animal died after approximately two weeks of exposure to natural forage and water in an area that had elevated levels of selenium. No sheep deaths or signs of illness were reported in the remaining HiSe group of sheep after four weeks of exposure to selenium forage and water levels in the range that would be considered toxic from other studies (Raisbeck, 2000; Davis et al., 2002). These animals had selenium levels in some tissues (blood, liver, kidney and skeletal muscle) that would be diagnostic of exposure to toxic levels of selenium (Blodgett and Bevill, 1987; Puls, 1997; Smith et al., 1999). High selenium concentrations were found in the liver of the ewe and lamb in the HiSe group (5.0 to 6.0 ppm). High selenium concentrations were also present in the kidney of the HiSe lamb (4.0 ppm) and diagnostic toxic levels of selenium exposure were present in the skeletal muscle of the HiSe ewe and lamb (0.7 to 1.4 ppm). No histopathological lesions in these animals were consistent with selenium toxicity. These results indicate some sheep are more susceptible to selenium toxicosis, which has also been

reported by others (Kuttler et al., 1961; Glenn et al., 1964c; Smyth et al., 1990). A panel of serochemistries (calcium, chloride, creatinine, potassium, sodium, urea, albumin, globulin, total bilirubin, total carbon dioxide, total protein, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase) and complete blood counts (eosinophil, hematocrit, hemoglobin, monocyte, platelet, red blood cells and white blood cells) also did not indicate any consistent clinical signs of serious health effects related to selenium toxicity. However, it has been reported in literature that serum activity in creatine kinase, aspartate aminotransferase, along with blood glucose and urea will increase as a result of *Astragalus* poisoning, a known selenium accumulator species (Quazzani et al., 1999). In the HiSe group, only aspartate aminotransferase showed elevated levels. Despite this, the only clinical observation seen in the HiSe group was an initial partial aversion (about 10 days) to forage and water at the beginning of the exposure phase that resulted in a slight decrease in body condition by the end of the study.

The 24 sheep from the LoSe group were missing from the study enclosure during the fourth week of the exposure phase. Twenty-two of these sheep were found on a different area of the mining site near the end of the study (21 days after completing the exposure phase, day 70). It is unknown exactly where these sheep were during the time they were missing. However, the selenium levels in the whole blood and serum of these animals indicate they had been exposed to elevated levels of selenium when compared to the Con group. The fate of the two sheep missing from this group is unknown but could be due to selenium toxicity, predation or natural causes. It is interesting that 22 of the 24 sheep in this group apparently survived in this area. Together, these results indicate there is individual variability to selenium tolerance in sheep. Variability of selenium tolerance in sheep has also

been reported by others under more controlled conditions which mainly tested inorganic selenium compounds (Kuttler et al., 1961; Glenn et al., 1964c; Smyth et al., 1990). It is apparent that more research is needed on the toxicology of selenium in sheep to better determine exposure levels which can be diagnostic of toxicosis, especially in a natural habitat and with the organic forms of selenium. Current diagnostic values in forage and tissues (including blood and serum) are often based on specific cases or incidences which typically involve specific exposure conditions, different forms of selenium, different breeds of sheep and usually relatively small numbers of animals (Glenn et al., 1964c; Civil and McDonald, 1978; Hopper et al., 1985; Blodgett and Beville, 1987; Smyth et al., 1990).

Sheep mortality in our study was 4% (1/24) in the HiSe group and 8% (2/24) in the LoSe group (if both missing sheep were assumed to die from selenium exposure in a worst case scenario). A mortality of 13% was reported in a previous incidence of 200 sheep deaths out of 1500 sheep grazing in the area and diagnosed with selenium toxicity. Another significant incident that occurred less than a year after the present study involved the death of 327 sheep in a band of 1400 sheep grazing this area (23%). The cause of death was diagnosed as selenium toxicosis by the veterinary toxicologist and pathologist at the Washington Animal Disease Diagnostic Laboratory. It is apparent that the grazing sheep in these reclaimed mining areas will result in the death of some sheep from selenium toxicosis and the owners should expect these losses. It seems from the data collected from our study and other episodes that the magnitude of the problem will vary from year to year and may be based on a combination of management (nutrition, stress, etc.), exposure (magnitude, duration, etc.) and environmental (temperature, precipitation/runoff, etc.) factors which we cannot yet accurately predict.

Exposure conditions can be semi controlled by identification of high selenium areas of forage and water, and avoidance of these areas. Also, clean water with non toxic selenium levels may be provided to grazing sites. The comparison of selenium levels of whole blood and serum between the HiSe and LoSe groups in our study indicate that sheep consuming high selenium forage but low selenium water accumulated lower selenium residues (blood and tissues) and therefore should be at less risk and could possibly be held on the site longer.

It became apparent following the episode of sheep deaths in May of 2003, a year after our study, that there are areas on these mining sites that have higher selenium levels than others. These “hot” areas of selenium can contain species of plants that can accumulate very high levels of selenium. For example, the *Aster chilensis* plants analyzed in the area where the sheep died in 2003 had selenium levels of 8000 ppm and the *Grindelia squarrosa* (curly-cup gumweed) plants had levels of 200 ppm. Plants in our study area of 2002 did not have these high levels of selenium. It seems the uneven distribution of selenium and selenium accumulating plants on these sites may explain the sporadic episodes that result in large numbers of sheep deaths in some years and not others. It may be entirely dependent on where the sheep are grazed on these sites during a particular grazing season.

Certain management practices may lower the risk of exposure to selenium. These management practices include analysis and mapping of high selenium areas and accumulator plants. Producers could learn the identity of seleniferous plants and identify grazing areas with lower selenium toxicity risks. Seleniferous plant identification may assist in avoiding areas that contain high concentrations of these plants. Alternatively, areas of high risk may fence or signed off to avoid accidental grazing (Davis et al., 2002). Another management practice that has been reported to reduce selenium toxicity mortalities is high protein diets. It

has been found that a high protein diet has decreased the recovery period after acute selenium intoxication, and increases the tolerance of sheep to selenium intoxication (Rosenfeld and Beath, 1946; Gardiner, 1966; Palmer et al., 1980). A high protein diet may have been a contributing factor in the HiSe group and their tolerance to toxic selenium exposures. An elevated level of serum urea in the HiSe sheep indicates a high protein diet, which may have increased the tolerance of these sheep to selenium. A balanced mineral mix that contains sulfur and copper has also been found to be beneficial in counteracting a high selenium diet and decrease the accumulation of both copper and selenium in tissues, along with their potential toxic effects in sheep (Ryssen et al., 1998; Davis et al., 2002). Decreasing the stocking rate and grazing a variety of forage types will assist in providing less competition for high quality forage and a balanced diet for the sheep (Schoacht et al., 1996; Duncan and Gordon, 1999; Greene and Fultz, 2002). The methods of handling of animals may also influence their susceptibility to selenium. Lowering the stress associated with handling during herding and transportation to and from grazing areas can increase the overall well-being and production performance (e.g., weight gain) of the animals (Gabbedy, 1970; Ewer, 1974; Grandin, 1997). Harsh environmental conditions such as hot or cold temperatures and feed or water deprivation and hands-on handling and poor grazing conditions may also contribute to increased stress in animals (Stott, 1981; Webster, 1983). Environmental stressors can be partially resolved by providing artificial or natural shelters and readily access to feed and water.

Differences between the ewes and lambs in whole blood selenium levels, serochemistry (albumin, alkaline phosphatase, calcium, globulin, total bilirubin, total protein and urea) and complete cell counts (platelets) were reported throughout the study. Whole

blood selenium levels in the HiSe group were higher in the lambs than the ewes. Since lambs require more nutrients (forage) for growth and have a higher rate of metabolism than ewes, higher selenium concentrations in their blood may have resulted from increased seleniferous forage consumption (Sheep Production Handbook, 1997). In the LoSe and Con groups, no significant differences were seen in the whole blood selenium levels between lambs and ewes. The lack of significance between the ewes and lambs of the LoSe and Con groups may have resulted from lower selenium exposures to both groups.

Serum albumin concentrations in all lambs were higher than the ewes in all groups but the globulin concentrations were higher in the ewes than their lambs. This condition has been reported in sheep that are parasitized by *Haemonchus* organisms (Coles, 1986). Gross necropsies conducted on ewes and lambs from the HiSe and Con groups revealed the presence of parasites. Since ewes are more mature than lambs they may have accumulated higher numbers of parasites than the lambs resulting in lower albumin and higher globulin concentrations. Serum alkaline phosphatase concentrations in all lambs were higher than the ewes. Younger animals typically have 2 to 3 times higher levels of serum alkaline phosphatase than adult animals since they are still developing and growing bones (Kaneko, 1989). Calcium levels were higher in all lambs than the ewes. Higher serum calcium levels in the lambs may result from higher metabolism rates in growing animals versus adult animals (Coles, 1986; Kaneko, 1989). Total bilirubin levels were higher in all lambs than their corresponding ewes. Bilirubin is the chief bile pigment found in the serum of domestic animals (Coles, 1986). Significant increases in total bilirubin are typically associated with a hemolytic crisis (Kaneko, 1989). However, since the elevation of bilirubin concentrations were within the normal range and increases were only seen in the lambs, it is speculated that

lambs may have higher concentrations than ewes due to a higher metabolism rate and increased forage consumption. Total protein levels were higher in all ewes than their corresponding lambs. Total protein levels are reported to be associated with total albumin and globulin concentrations. Higher protein levels in the ewes may have resulted from a decrease in albumin and an increase in globulin concentrations (Coles, 1986). Urea concentrations in all lambs after day 28 were higher than their corresponding ewes. Since urea is one of the principle end products of protein catabolism, higher levels are expected to occur in lambs due to their higher protein turnover rate than ewes (Coles, 1986). However, since selenium is typically associated with proteins (e.g., selenomethionine), higher levels in the HiSe group may relate to higher selenium exposures in forage and water (Schrauzer, 2000; Wright et al., 2002). Platelets counts were higher in all lambs than the ewes. Platelets are involved with the blood vessel wall and the contact-activated coagulation factors in the initiation of the hemostatic process (Coles, 1986; Kaneko, 1989). These counts may be increased in lambs due to growing changes and platelet turnover. However, in ewes, decreased counts may relate to decreased production of platelets (typically associated with an older ewe) and a slower turnover rate (Kaneko, 1989).

Sheep in the HiSe group were exposed to selenium in forage and drinking water that has been reported in literature to be toxic (Puls, 1994; Davis, 2002). However, no gross, clinical or histopathological signs of selenium toxicity or other illnesses and effects on conception were observed in the HiSe sheep. Selenium toxicity is known to have effects on conception and the results from the pregnancy analysis in the HiSe group indicated no responses from selenium exposure. Sheep require approximately 2.6 lbs of forage per day dry intake for maintenance, based on a 154 lb ewe. A ewe will drink approximately 1.5

gallons of water per day (Sheep Production Handbook, 1997; Umberger, 2001). Forage available to the HiSe group averaged 14.2 ppm selenium and the drinking water averaged 375 ppb selenium. A rough calculation of selenium exposure to the HiSe sheep was therefore estimated at 0.26 mg Se/kg body weight. This calculation of selenium exposure is an estimation based on a mean of all plant and water selenium concentrations reported in the HiSe pen. However, selenium concentrations in the HiSe sheep blood (whole blood and serum) (Figure 4.1.1 and 4.1.2) and tissues of a ewe and lamb (Table 4.4.1 and 4.4.2) confirm that selenium accumulated in their tissues to levels considered to be toxic. These reference values are based on reported cases of selenium toxicity. They do not account for the form of selenium, dose, age, sex and duration of exposure. The exposure in this present study is significantly lower than a LD_{50} for sheep that was reported at 0.46 mg Se/kg body weight after intramuscular injections of sodium selenite (Combs and Combs, 1986; Blodgett and Bevill, 1987). It is however in a range where some deaths may occur.

A continuation of this research project with increased numbers of sheep and samples (e.g., blood, urine, feces, plants, soils and water) may provide additional information to prevent loss of sheep grazing these areas. Additional necropsies and analysis of urine, fecal and tissue levels of selenium would create a more accurate assessment of natural selenium exposure and prediction of toxicity. Testing grazing sites throughout reclaimed mine areas on various terrains and forage plant species may also result in more information about recent sheep deaths. Also, testing sheep grazing forage high in selenium but providing low selenium water may further provide further information that sheep receiving low selenium water can tolerate high selenium forage for a longer period of time. No other studies have

been conducted with animals grazing natural selenium exposure. The natural setting makes this study unique in its testing environment and selenium exposure.

In summary, reclaimed phosphate mining sites in southeastern Idaho contain elevated levels of selenium in the soil, water and plants that can result in mortalities of animals. Significant numbers of sheep deaths (> 500) have been attributed to grazing areas that were high in selenium. Although elevation in the blood (whole and serum) and tissues (kidney, liver and skeletal muscle) were reported in the HiSe group, only one sheep death resulted from clinically diagnosed selenium toxicity. This particular ewe that died had lower selenium concentrations than both a ewe and lamb from the HiSe group that were necropsied and showed no gross, histopathological or clinical signs of selenium toxicosis. Other studies have reported that the similar selenium levels found in the tissues and in the blood should have resulted in illness or deaths in the HiSe group besides the one ewe. Plants within the HiSe and LoSe pen contained selenium concentrations that have been reported toxic by numerous sources (Rosenfeld and Beath, 1964; National Research Council, 1980; Wilber, 1980; Combs and Combs, 1986; Puls, 1994; Sheep Product Handbook, 1997). The LoSe group disappeared before the completion of the exposure phase. The LoSe group sampled at the end of the study had elevated levels of selenium in their blood that was similar to the HiSe group. This suggests that these sheep were exposed to selenium levels and conditions that were similar to the HiSe group. Two animals from the LoSe group were never recovered and assumed dead. However, no illness or other cases of selenium toxicity were observed in any sheep (HiSe, LoSe and Con). These observations suggest that additional selenium research is needed to determine the susceptibility of sheep grazing naturally seleniferous areas. A baseline of what constitutes selenium toxicity and resulting mortality needs to be

established for this particular environment. Also attention to best management practices may minimize the number of fatalities and relationship between environmental conditions/stress should be monitored as a contributing factor. Until the problem is better defined, owners should expect some mortality of sheep grazing these areas and the magnitude of sheep deaths will vary unpredictably from year to year. The recent deaths of 327 sheep in this region appeared to be a result of consuming plants with very high selenium levels. This indicates that some animals were on these main sites which may have higher levels of selenium than others in soil and plants. Therefore, it is very important to know where these areas are in the mine sites and avoid grazing them. The sporadic distribution of selenium most likely is the reason that these poisoning episodes have been unpredictable from year to year.

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7.0 Appendix

7.1 Serochemistries within the Normal Reference Range

Chloride is a major extracellular anion. Most chloride is absorbed and in excess is excreted in urine. Low chloride levels may be seen with gastrointestinal (HCl) losses, diabetic ketoacidosis, mineralocorticoid excess and salt-losing renal diseases. Low serum values may also be encountered with compensated respiratory acidosis and metabolic alkalosis. High levels of chloride may occur during metabolic acidosis resulting from excess loss of bicarbonate due to losses from the lower gastrointestinal tract in diarrhea, renal tubular acidosis and mineralocorticoid deficiency (Henry, 1996; Kaneko, 1986).

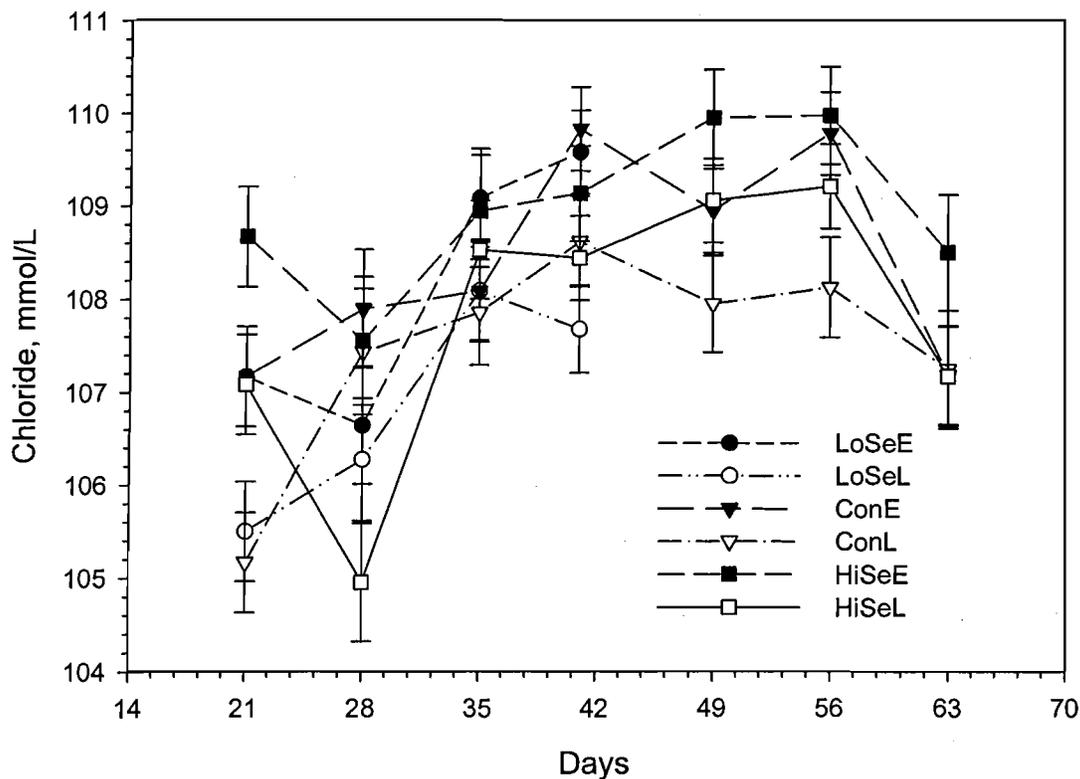


Figure 7.1.1 Serum chloride levels in sheep (mean \pm standard error).

Normal Range: 100.8 – 113.0 mmol/L

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.1.1 *Level of significance (< 0.05) of serum chloride levels in sheep. Control Lamb (CL) – High Lamb (HL)*

Days	Level of Significance < 0.05
21	CL – HL
28	CL - HL

Potassium is a major intracellular cation. Potassium is coupled with the active extrusion of sodium from the cells, which is maintained by the sodium-potassium pump. The movement of potassium across the cell membrane plays a critical role in the maintenance of cardiac and neuromuscular excitability. Decreased serum levels or hypokalemia may occur even when the total amount of potassium in the body is normal. Depletion of potassium stores occur as a result of gastrointestinal fluid losses due to vomiting or diarrhea or renal losses. This condition results in an increase in membrane potential, producing a hyperpolarization block resulting in weakness or paralysis. Increased serum levels or hyperkalemia may result from an increase in total body potassium. Hyperkalemia decreases membrane potential causing hyperexcitability. This change may occur from acidemia and from cellular damage (e.g., fever, hemolysis, rhabdomyolysis). An increase in potassium levels typically occur in acute and chronic renal failure and in mineralocorticoid deficiency (Henry, 1996; Kaneko, 1986).

Increases in sodium content may result in expansion of extracellular fluid that eventually may lead to the development of hypertension or edema formation. Sodium depletion results most often from gastrointestinal losses through vomiting or diarrhea. Sodium losses also occur frequently from the presence of generalized chronic renal diseases (Coles, 1986). However, sodium excess often results from an increase in body water leading to an isotonic expansion of extracellular fluid volume and the development of hypertension

or generalized edema. Congestive heart failure, hypoalbuminemia, and hepatic fibrosis may lead to a failure to maintain effective circulating volume which can in turn result in compensating renal sodium retention (Kaneko, 1989).

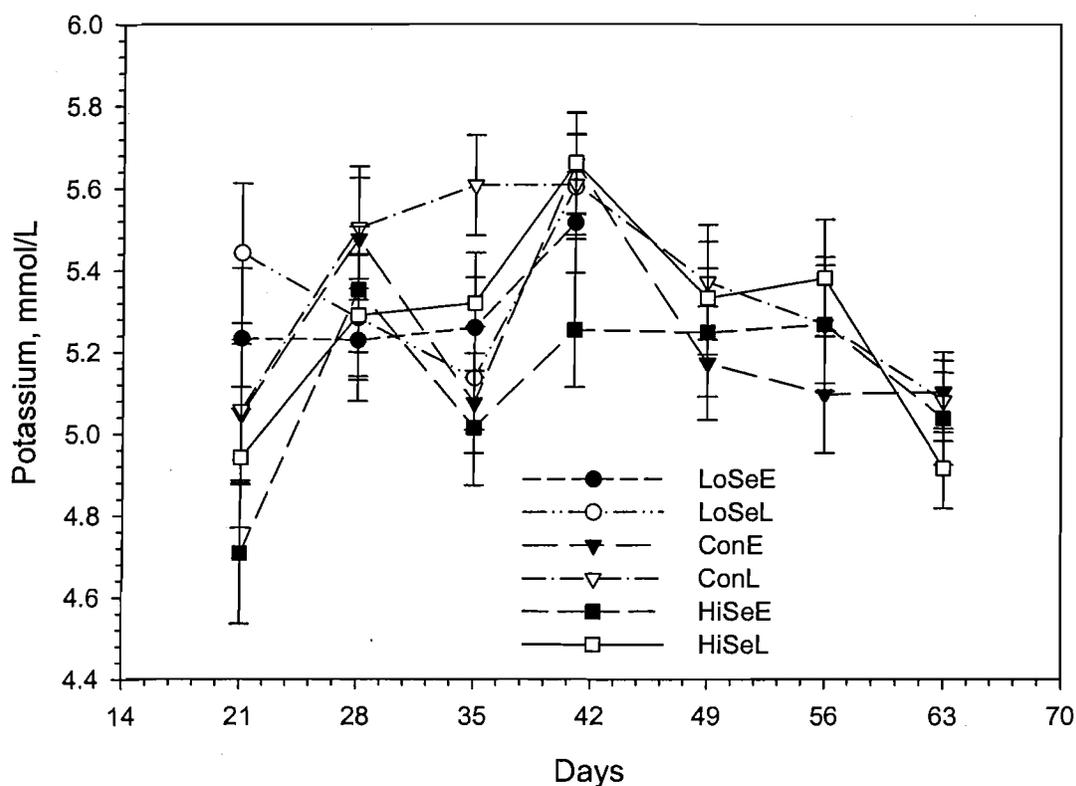


Figure 7.1.2 Serum potassium levels in sheep (mean \pm standard error).

Normal Range: 4.3 – 5.6 mmol/L

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.1.2 Level of significance (< 0.05) of serum potassium levels in sheep.
Control Ewe (CE) – Control Lamb (CL) – High Ewe (HE) – Low Lamb (LL)

Days	Level of Significance < 0.05
35	CL – LL
42	CE – HE

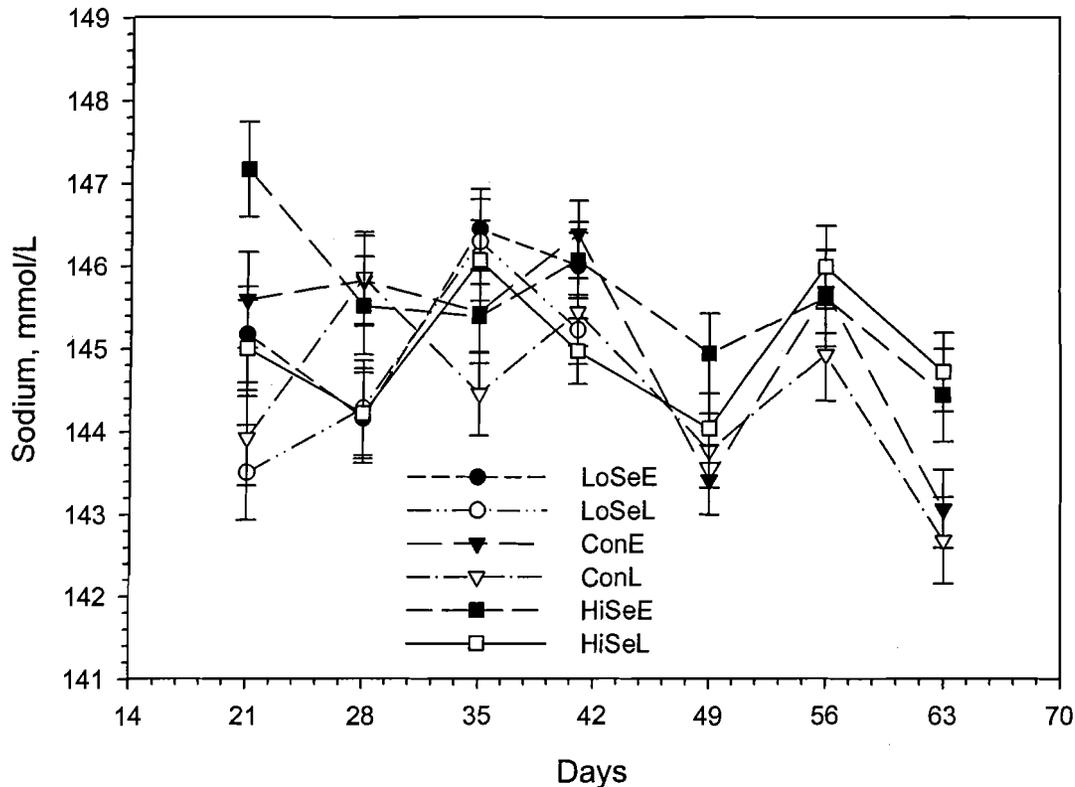


Figure 7.1.3 Serum sodium levels in sheep (mean \pm standard error).

Normal Range: 143 – 151 mmol/L

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.1.3 Level of significance (< 0.05) of sodium levels in sheep.

Control Ewe (CE) – Control Lamb (CL) – High Ewe (HE) – High Lamb (HL) – Low Ewe (LE) – Low Lamb (LL)

Days	Level of Significance < 0.05
28	CL – HL; CE – LE; CL – LL
35	CL – HL; CL – LL
49	CE – HE
63	CL – HL

Eosinophils are phagocytic and may ingest bacteria, fungi, mycoplasmas, inert particles and antigen-antibody complexes *in vitro*. These cells participate in several immune and inflammatory activities. They also are antiparasitic (accumulate in tissues as a response

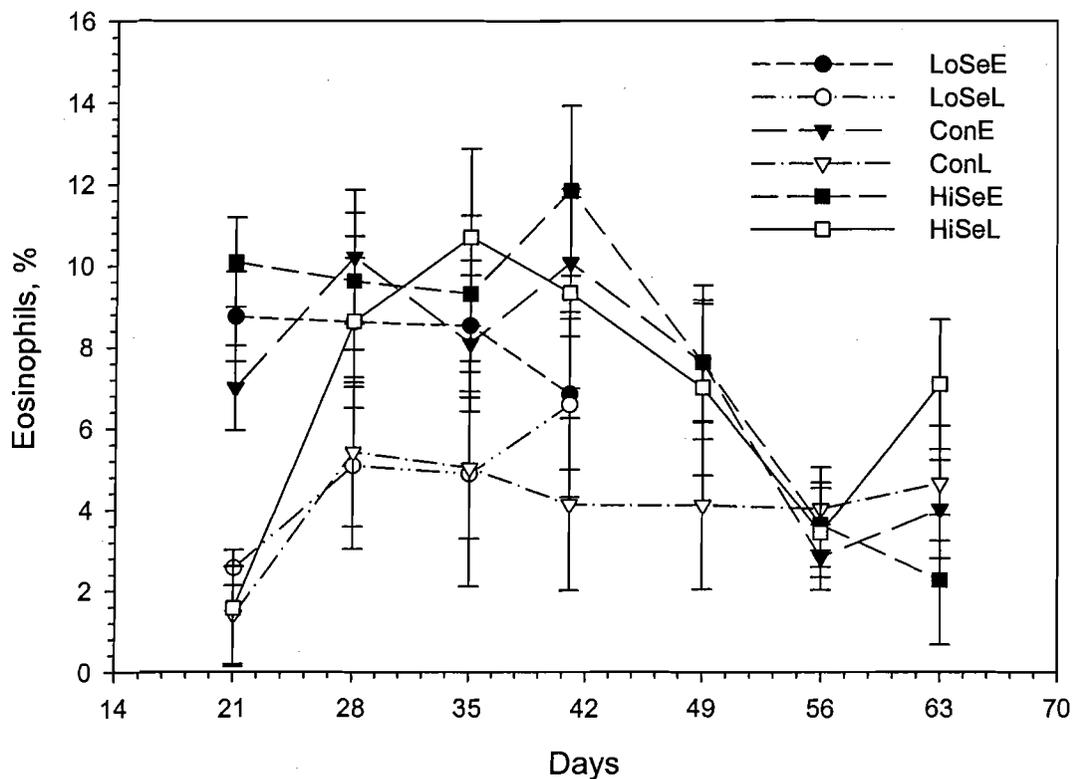


Figure 7.1.4 Serum eosinophil levels in sheep (mean \pm standard error).

Normal Range: 0 – 10%

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.1.4 Level of significance (< 0.05) of serum eosinophil levels in sheep.

Control Lamb (CL) – High Lamb (HL)

Days	Level of Significance < 0.05
35	CL – HL

from parasites) and modulate the inflammatory and immediate hypersensitivity reactions and may damage tissues in certain types of hypersensitive reactions (Coles, 1986; Kaneko, 1989; Mean and Harvey, 1998).

Hemoglobin is the main component of the red blood cells. It is a conjugated protein that serves as the vehicle for transportation of oxygen and carbon dioxide. The main function

of hemoglobin is to transport oxygen from the lungs, where oxygen tissue is high to tissues where oxygen is low. A decrease to below normal hemoglobin concentration, erythrocyte count or hematocrit is a common condition and may be a sign of anemia. These low counts are also frequently a complication of other diseases (Henry, 1996).

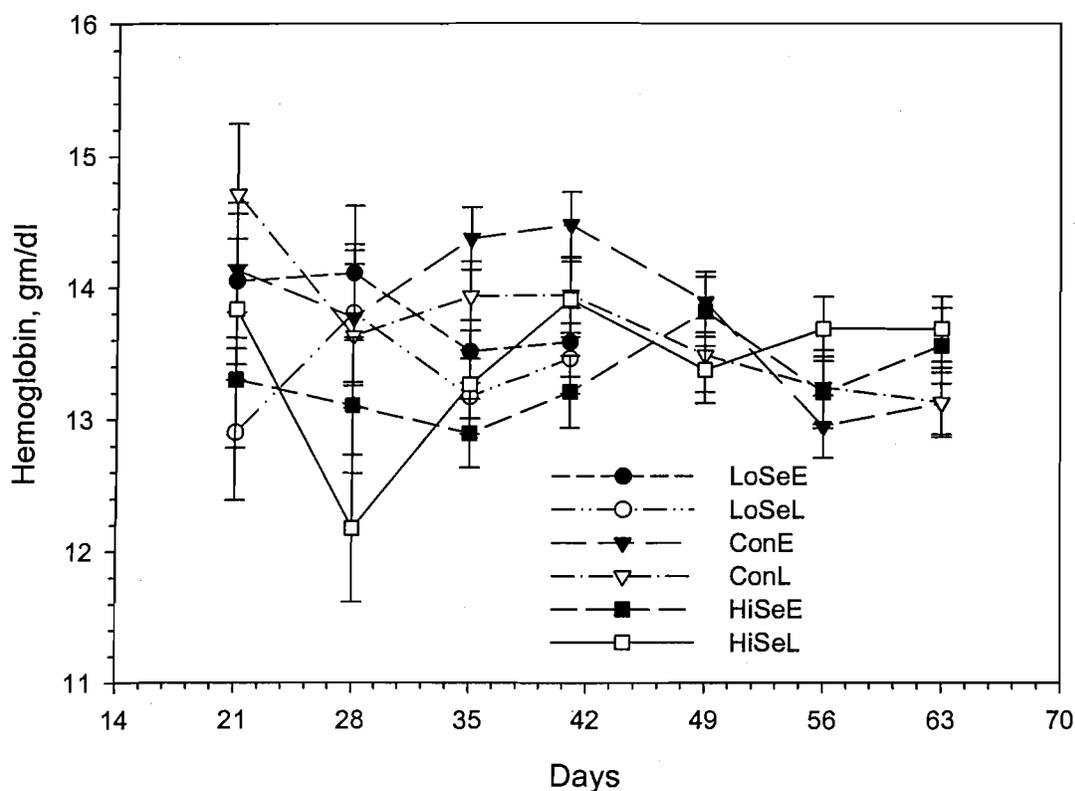


Figure 7.1.5 Hemoglobin levels in sheep (mean \pm standard error).

Normal Range: 8 – 16 gm/dl

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.1.5 Level of significance (< 0.05) of hemoglobin levels in sheep.

Control Ewe (CE) – Control Lamb (CL) – High Ewe (HE) – High Lamb (HL) – Low Ewe (LE)

Days	Level of Significance < 0.05
21	CL – HL
35	CE – HE; CE – LE
42	CE – HE; CE – LE

Monocytes arise predominantly in the bone marrow. They are immature cells that migrate into tissues and become a macrophage. Monocytes play an important role in inflammation by secreting biologically active substances (e.g., prostaglandins, proteolytic enzymes etc.) However the main function of monocytes is phagocytosis, by ingesting and removing large particles of cellular debris that accumulate in tissues (Coles, 1986; Kaneko, 1989). Increased levels of monocytes results in monocytosis. Monocytosis occurs during the recovering stage of acute infections and from agranulocytosis. Decreased levels of monocytes result in monocytopenia (Figure and Table 7.1.6) (Henry, 1996).

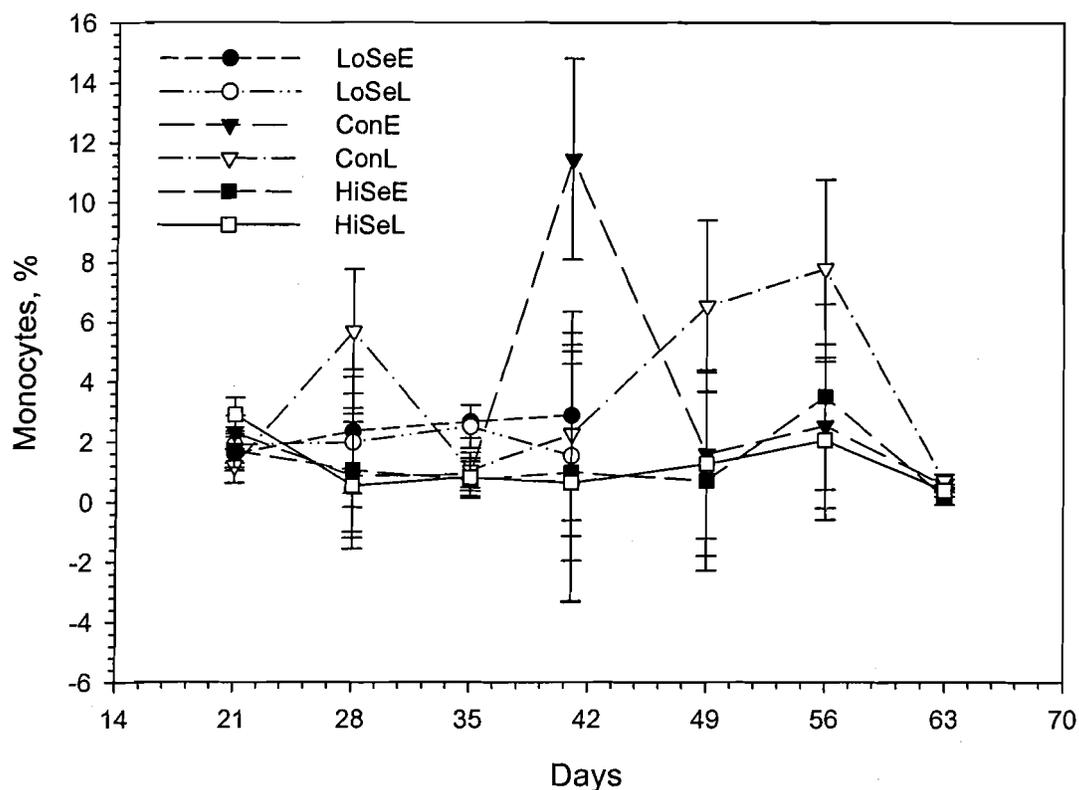


Figure 7.1.6 Monocyte numbers in sheep (mean \pm standard error).

Normal Range: 0 – 6%

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.1.6 *Level of significance (< 0.05) of monocytes numbers in sheep.*
Control Ewe (CE) – Control Lamb (CL) – High Ewe (HE) –
High Lamb (HL) – Low Ewe (LE) – Low Lamb (LL)

Days	Level of Significance < 0.05
21	CL – HL
35	CE – LE

Platelets are cellular particles produced by megakaryocytes mainly of the bone marrow. The lung and spleen are also sources of platelets. Platelets are involved with the blood vessel wall and the contact-activated coagulation factors in the initiation of the hemostatic process. A decrease in levels (thrombocytopenia) or presence of abnormal, nonfunctional will impair hemostasis. Excess levels may indicate inadequate hemostasis (Kaneko, 1986; Meyer and Harvey, 1998).

Red blood cells have three main functions that include transporting oxygen to tissues, transporting carbon dioxide to the lungs and buffering hydrogen ions. Reduced counts may result from anemia such as chronic renal disease, endocrine deficiencies and chronic disease (Meyer and Harvey, 1998).

White blood cells function in killing of microbes and the disposal of foreign substances. Functions of white blood cells are carried out by neutrophils, monocytes/macrophages and eosinophils. A condition called leukocytosis refers to an increase in the total white blood cells. A decrease in white blood cells is seen in leucopenia (Henry, 1996; Meyer and Harvey, 1998). However, decreased counts have been reported to occur in sheep as they mature, due to a fall in the number of lymphocytes (Coles, 1986).

Creatinine is a nonprotein nitrogenous substance formed during muscle metabolism of creatin and phosphocreatin. If production remains constant creatinine may provide a crude

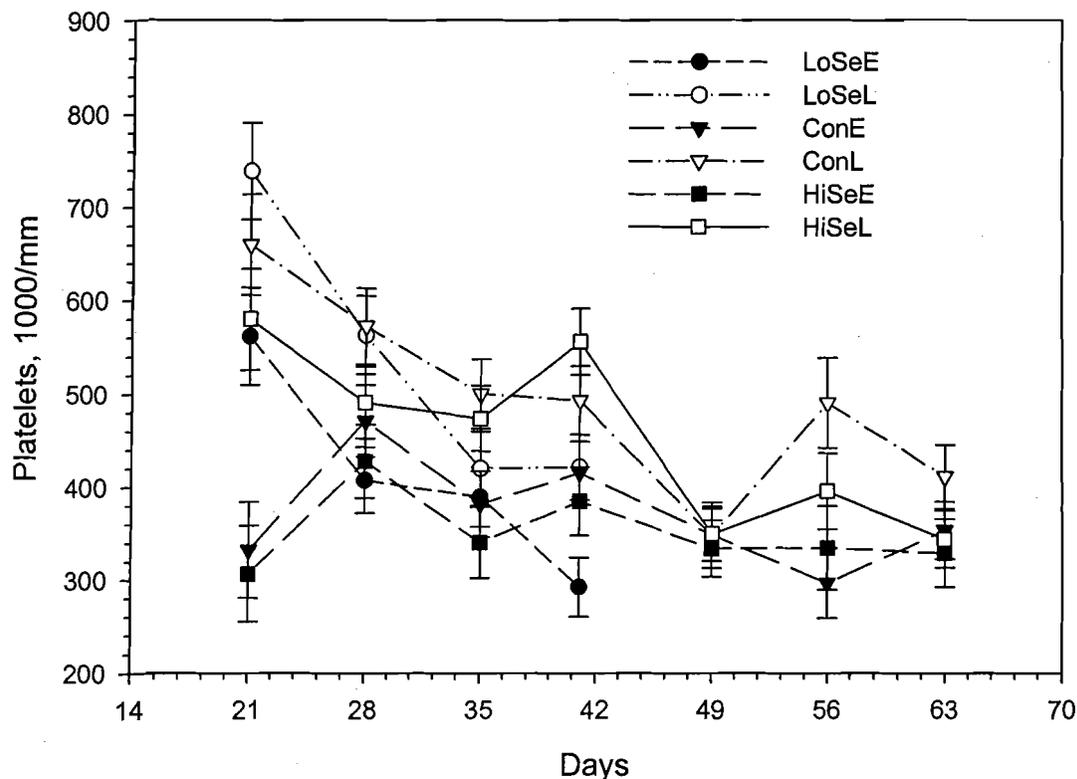


Figure 7.1.7 Platelet numbers in sheep (mean \pm standard error).

Normal Range: 250 – 750 (1000/mm)

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.1.7 Level of significance (< 0.05) of platelets numbers in sheep.

Control Ewe (CE) – Low Ewe (LE)

Days	Level of Significance < 0.05
21	CE – LE
42	CE – LE

measurement of glomerular filtration (Coles 1989). Serum creatinine is used as an indicator of the retention of nitrogenous wastes by the kidney. Quantity of creatinine formed each day depends on the total body content of creatine, which depends on dietary intake, rate of synthesis of creatine and muscle mass (Kaneko 1989).

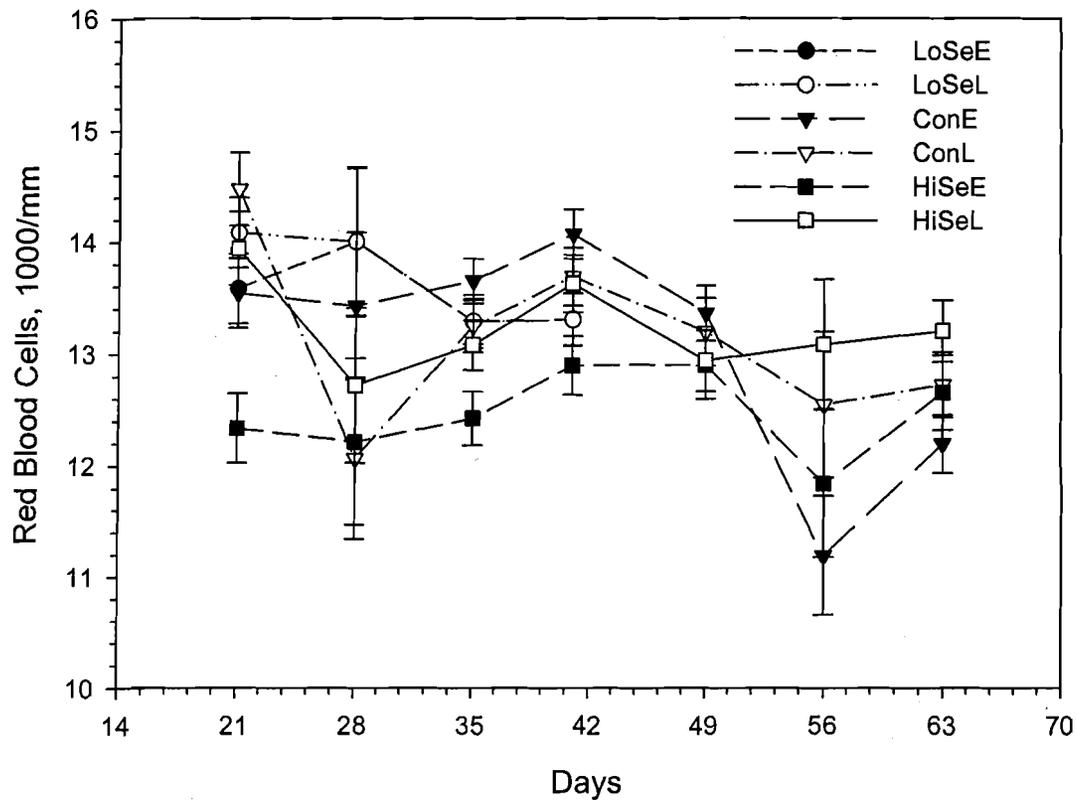


Figure 7.1.8 Red blood cell numbers in sheep (mean \pm standard error).

Normal Range: 8.0 – 16.0 (1000/mm)

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.1.8 Level of significance (< 0.05) of red blood cells numbers in sheep.

Control Ewe (CE) – Control Lamb (CL) – High Ewe (HE) – High Lamb (HL) – Low Ewe (LE) – Low Lamb (LL)

Days	Level of Significance < 0.05
21	CE – HE
35	CE – HE
42	CE – HE; CE – LE

Urea is formed within the liver and represents the principal end product of protein catabolism. It is reported to have no useful function within the body and is excreted mainly

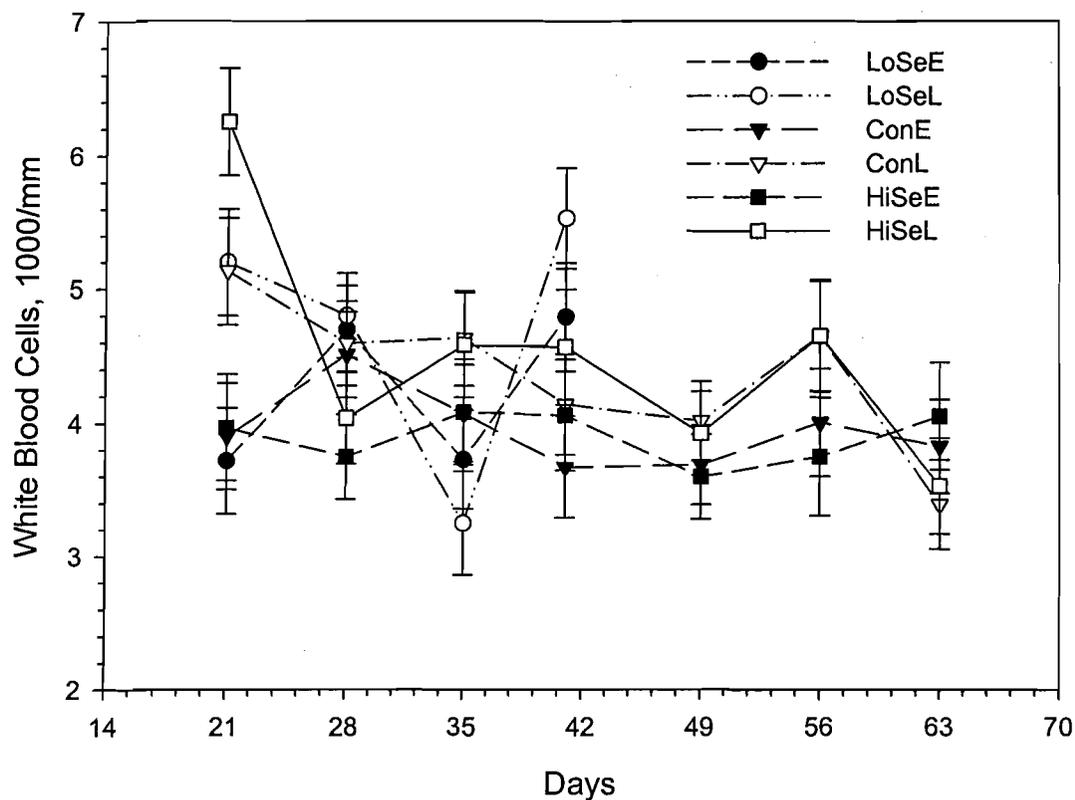


Figure 7.1.9 White blood cells numbers in sheep (mean \pm standard error).

Normal Range: 4 – 12 (1000/mm)

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.1.9 Level of significance (< 0.05) of white blood cells numbers in sheep.

Control Ewe (CE) – Control Lamb (CL) – Low Ewe (LE) – Low Lamb (LL)

Days	Level of Significance < 0.05
35	CL – LL
42	CE – LE; CL – LL

by the kidneys (Coles 1986). Elevated urea levels may result from diffuse liver disease which is a reduced capacity to synthesize urea (Kaneko 1989).

Total protein alterations may occur from a decrease in the quantity of albumin. This is accompanied by a relative hyperglobulinemia (Coles, 1986).

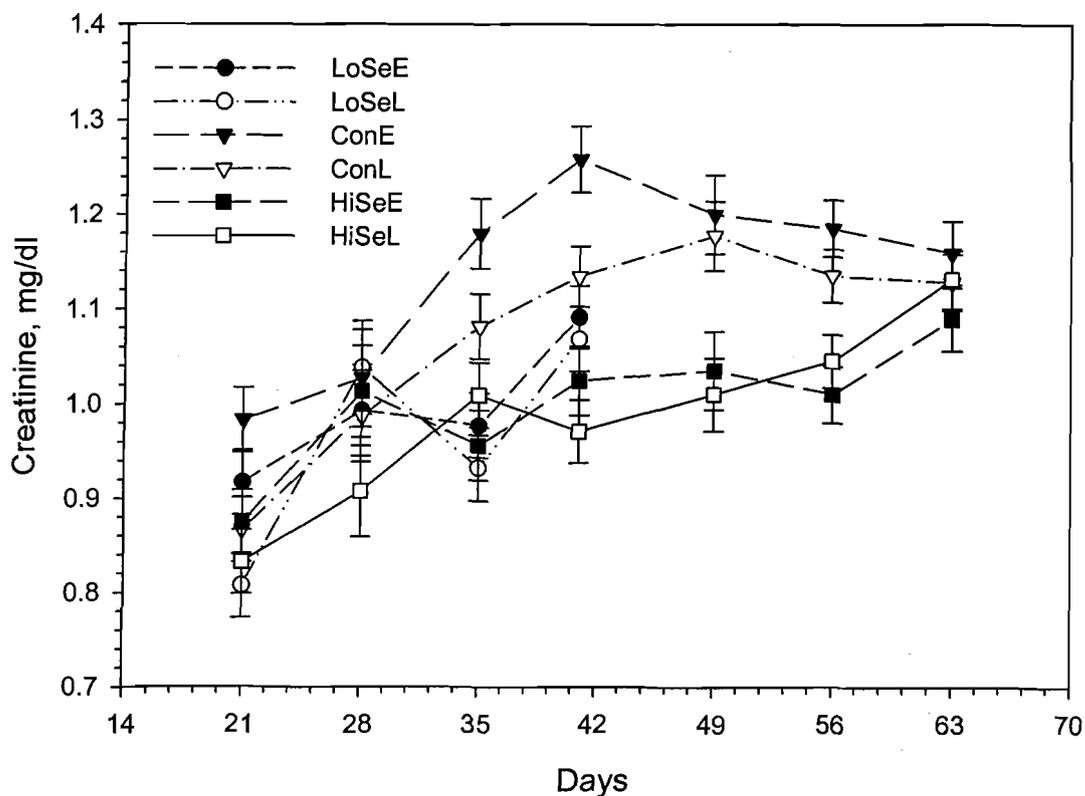


Figure 7.1.10 Serum creatinine levels in sheep (mean \pm standard error).

Normal Range: 0.7 – 1.3 mg/dl

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.1.10 Level of significance (< 0.05) of serum creatinine levels in sheep.

Control Ewe (CE) – Control Lamb (CL) – High Ewe (HE) – High Lamb (HL) – Low Ewe (LE) – Low Lamb (LL)

Days	Level of Significance < 0.05
21	CE – HE
35	CE – HE; CE – LE; CL – LL
42	CE – HE; CL – HL; CE – LE
49	CE – HE; CL – HL
56	CE – HE; CL – HL

Alkaline phosphatase is made up of a group of isoforms of nonspecific enzymes which hydrolyze many types of phosphate esters whose natural substrate or substrates are

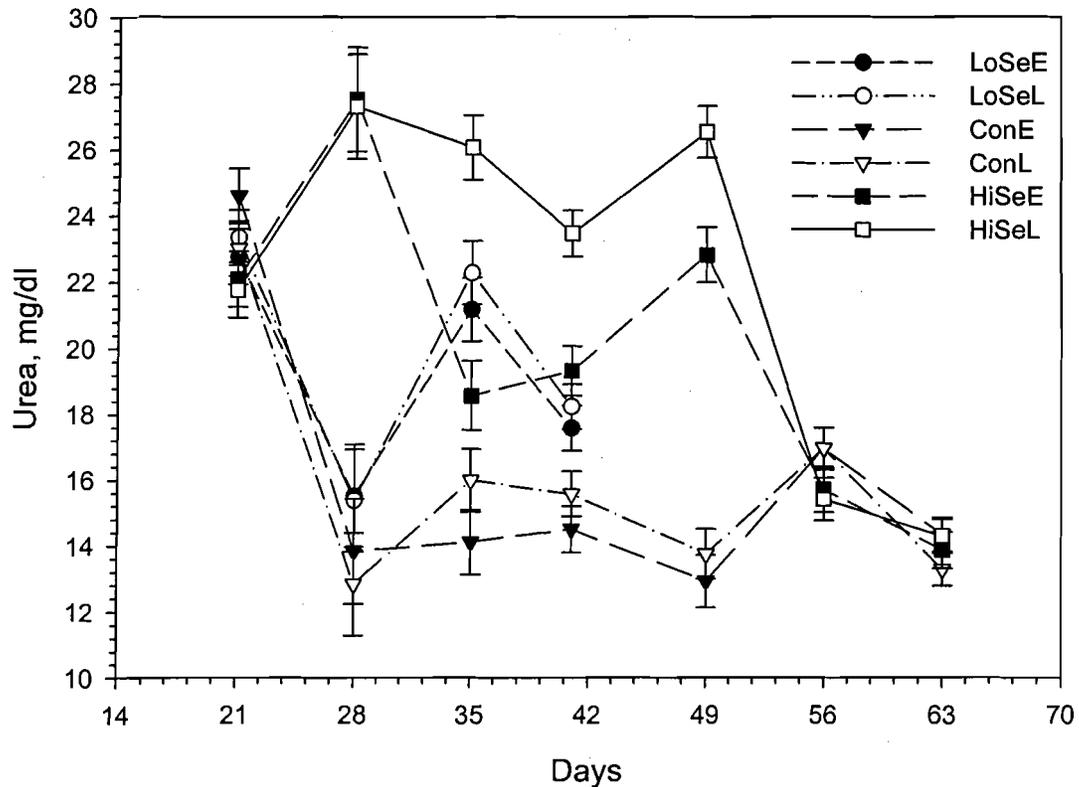


Figure 7.1.11 Serum urea levels in sheep (mean \pm standard error).

Normal Range: 14 – 27 mg/dl

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.1.11 Level of significance (< 0.05) of serum urea levels in sheep.

Control Ewe (CE) – Control Lamb (CL) – High Ewe (HE) – High Lamb (HL) – Low Ewe (LE) – Low Lamb (LL)

Days	Level of Significance < 0.05
21	CE – HE
28	CE – HE; CL – HL
35	CE – HE; CL – HL; CE – LE; CL – LL
42	CE – HE; CL – HL; CE – LE; CL – LL
49	CE – HE; CL – HL

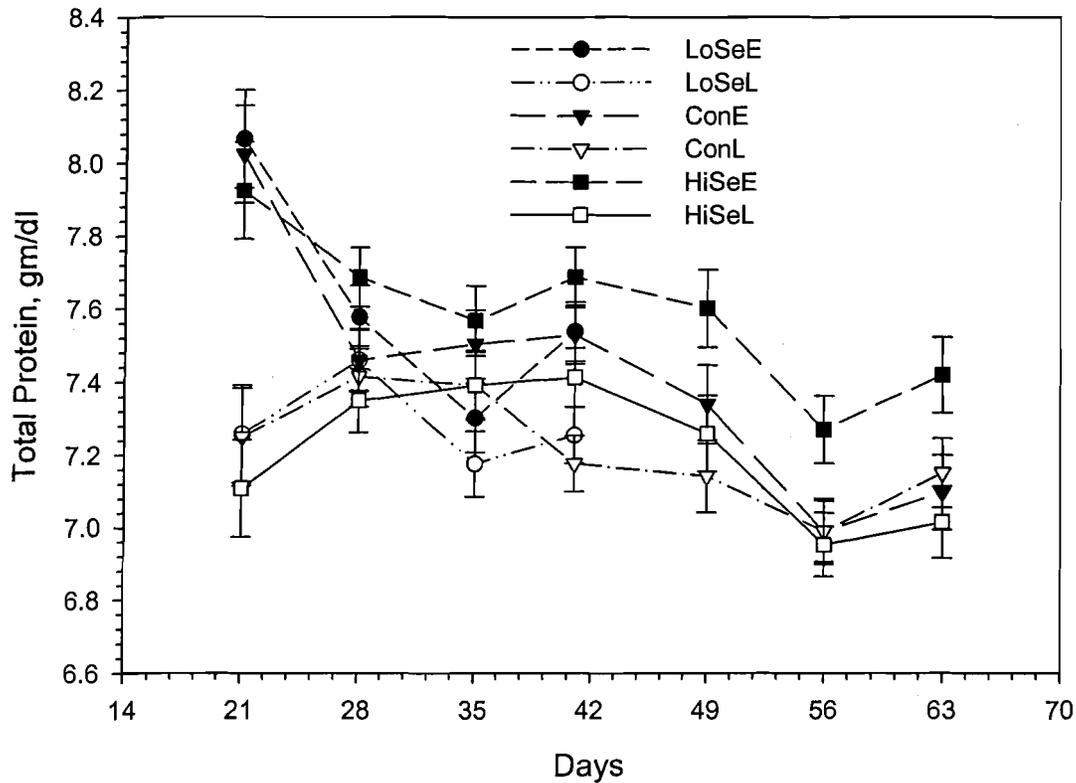


Figure 7.1.12 Total protein levels in sheep (mean \pm standard error).

Normal Range: 7 – 9 gm/dl

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.1.12 Level of significance (< 0.05) of total protein levels in sheep.

Control Ewe (CE) – Control Lamb (CL) – High Ewe (HE) – High Lamb (HL) – Low Ewe (LE)

Days	Level of Significance < 0.05
28	CE – HE
42	CL – HL
56	CE – HE
63	CE – HE

unknown. Alkaline phosphatase levels may increase in cases of bone and liver diseases and can be used as indicator for these disorders. A decrease in these levels is reported in mature

or maturing animals. Total serum activity is reported at 2 or 3 times greater in younger animals than adults (Kaneko 1989).

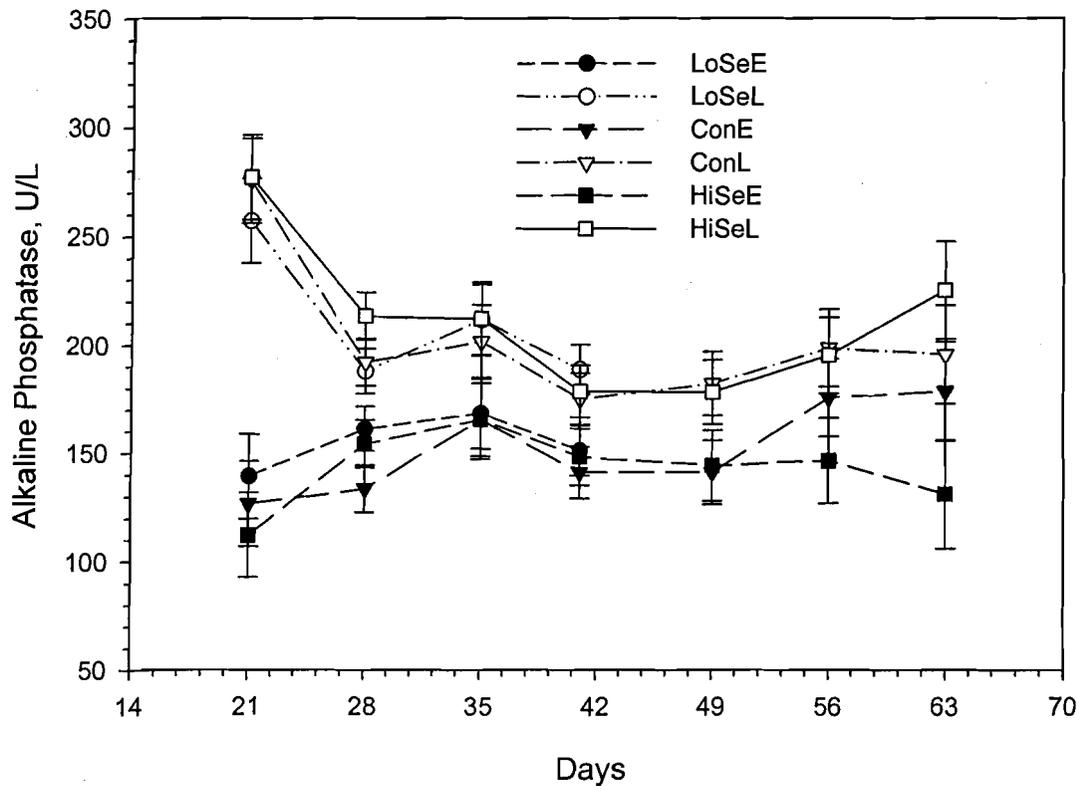


Figure 7.1.13 Serum alkaline phosphatase levels in sheep (mean \pm standard error).

Normal Range: 52 – 274 U/L

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.1.13 Level of significance (< 0.05) of serum alkaline phosphatase levels in sheep. Control Ewe (CE) – High Ewe (HE)

Days	Level of Significance < 0.05
63	CE – HE

Serum alanine aminotransferase levels are a value that can detect liver diseases in the dog, cat and primate. Alanine aminotransferase is increased in serum when cellular

degeneration or destruction of the liver organ occurs (Coles 1986). However, alanine aminotransferase in tissues of pigs, horses, cattle, sheep or goats is too low to be of diagnostic value (Kaneko 1989).

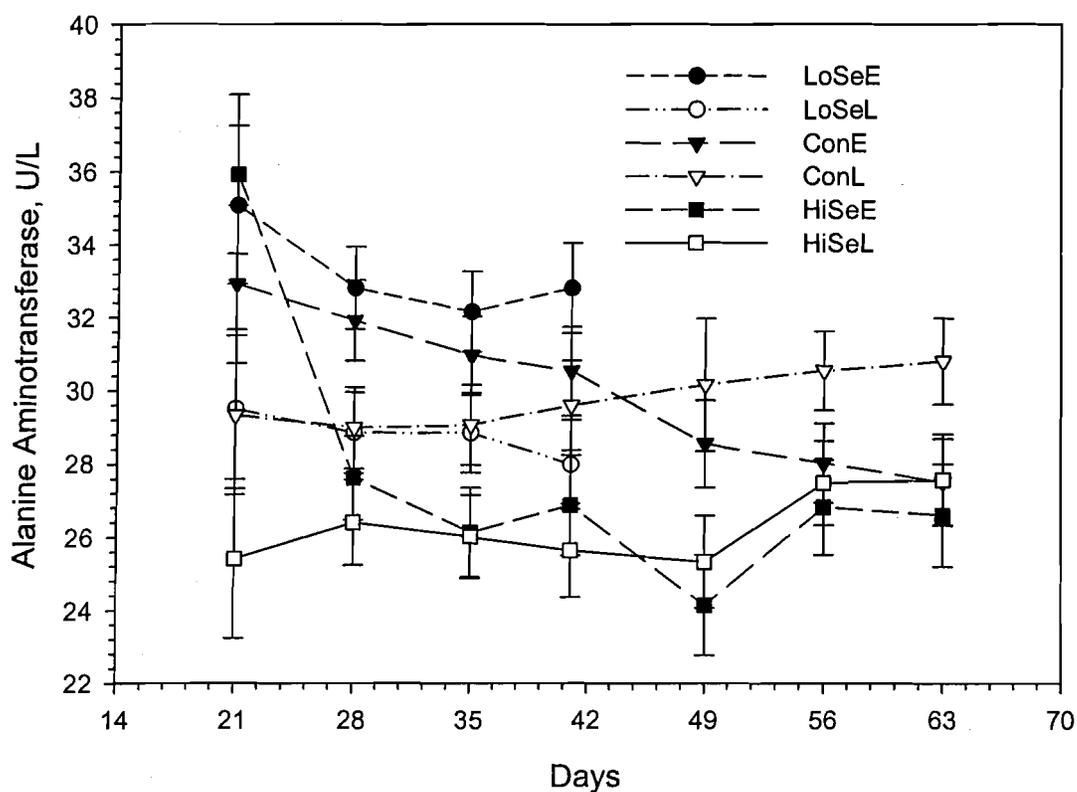


Figure 7.1.14 Serum alanine aminotransferase levels in sheep (mean \pm standard error).

Normal Range: 16 – 35 U/L

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.1.14 Level of significance (< 0.05) of serum alanine aminotransferase levels in sheep. Control Ewe (CE) – Control Lamb (CL) – High Ewe (HE) – High Lamb (HL) – Low Ewe (LE)

Days	Level of Significance < 0.05
28	CE – HE
35	CE – HE
42	CE – HE; CL – HL
49	CE – HE; CL – HL

Total bilirubin concentrations are directly proportional to its production from the rate of heme turnover and inversely related to hepatic clearance. Bilirubin clearance is dependent on the uptake of the liver and subsequent conjugation to glycosyl derivatives in hepatic microsomes. Normal bilirubin levels in sheep are less than 0.5 mg/dl (Kaneko 1989). However, normal bilirubin levels may have mean values between 0.10 and 0.19 mg/dl (Coles 1986). Major elevation in total serum bilirubin levels in sheep are usually observed only in hemolytic crisis. An increase and sustained level of direct-reacting bilirubin are indicative of either severe terminal hepatic disease or extrahepatic bile duct obstruction (Kaneko 1989).

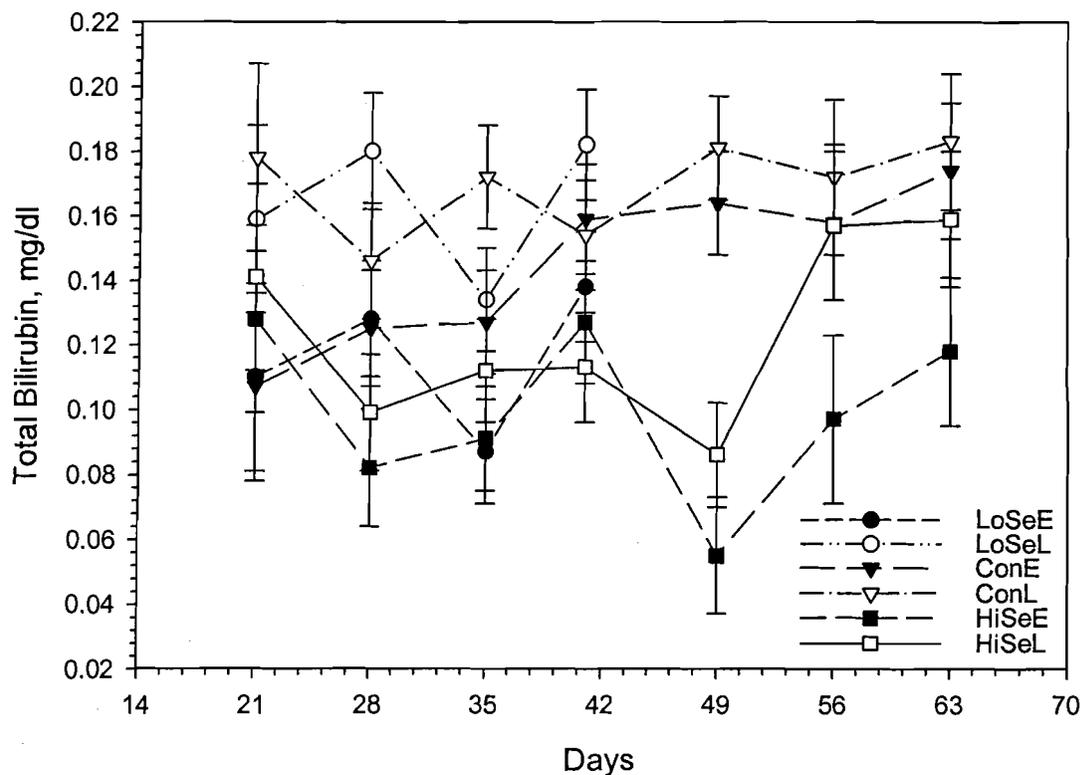


Figure 7.1.15 Total bilirubin levels in sheep (mean \pm standard error).

Normal Range: < 0.2 mg/dl

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.1.15 *Level of significance (< 0.05) of total bilirubin levels in sheep.
Control Ewe (CE) – Control Lamb (CL) – High Ewe (HE) –
High Lamb (HL) – Low Ewe (LE) – Low Lamb (LL)*

Days	Level of Significance < 0.05
35	CL – HL
49	CE – HE; CL – HL

7.2 Serochemistries Above the Normal Reference Ranges

Calcium is the most abundant cation in the body and is mainly stored in bone. The kidneys are important in the regulation of calcium levels. In renal failure, calcium levels fall as phosphate levels tend to rise. Hypocalcemia may be caused by alkalosis and renal failure but also hypoparathyroidism which may lead to hyperphosphatemia. Hypercalcemia may result from acidosis (Henry, 1996).

Hematocrit counts are typically interpreted with total protein concentrations. Splenic contraction, primary or secondary erythrocytosis or dehydration-masked hypoproteinemia may cause elevated hematocrit counts even when total protein concentrations are normal. It has also been reported that excitement or exercise immediately before sampling may also result in a 30-50% increase in hematocrit counts in cats, dogs and horses (Meyer and Harvey, 1998). A decrease in hematocrit counts may suggest a true or absolute anemia. This may result from blood loss, increased red blood cell destruction, or decreased red blood cell production (Meyer and Harvey, 1998).

Elevated globulin concentrations may result from bacterial infections, viral infections, parasitism or liver disease (Coles, 1986). See albumin for additional information.

Aspartate aminotransferase is present in many tissues and may be utilized in the detection of the destruction (i.e., necrosis) of many tissues (e.g., skeletal, cardiac or hepatic).

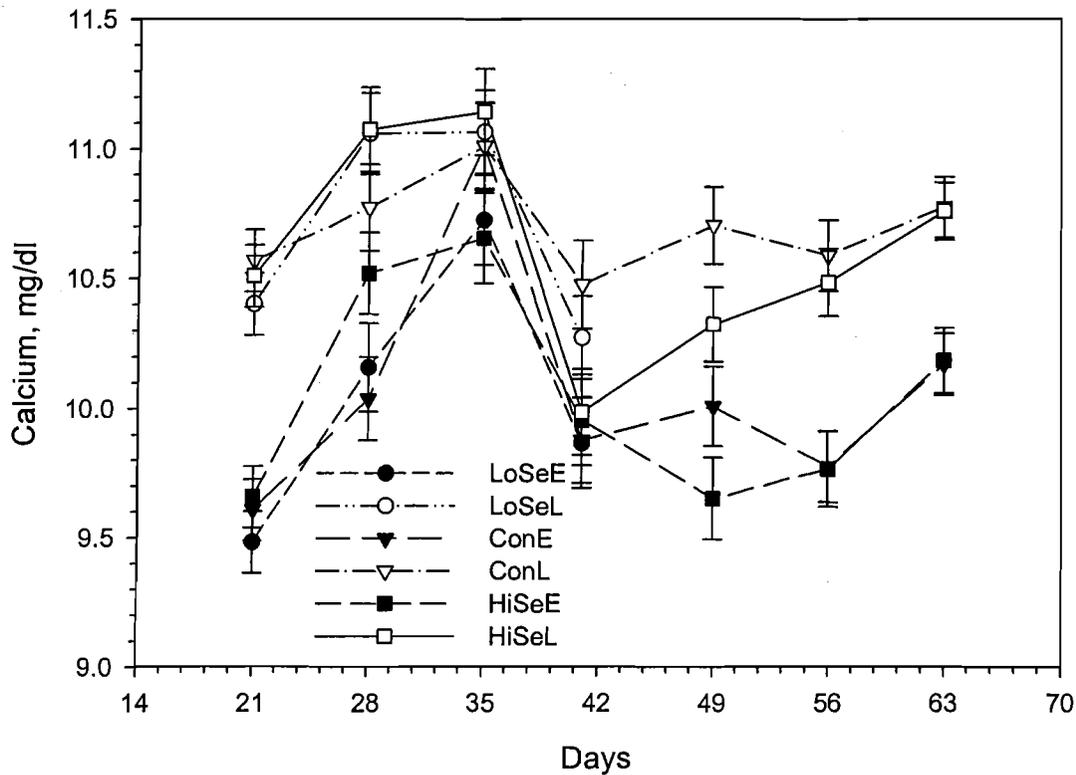


Figure 7.2.1 Serum calcium levels in sheep (mean \pm standard error).

Normal Range: 8.5 – 10.6 mg/dl

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.2.1 Level of significance (< 0.05) of serum calcium levels in sheep.

Control Lamb (CL) – Low Lamb (LL)

Days	Level of Significance < 0.05
42	CL – HL

Elevated levels have been observed in animals infested with liver flukes (Coles, 1986). It has also been suggested that aspartate aminotransferase may indicate muscle degeneration after creatinine levels have fallen; aspartate aminotransferase has a reportedly longer half-life than

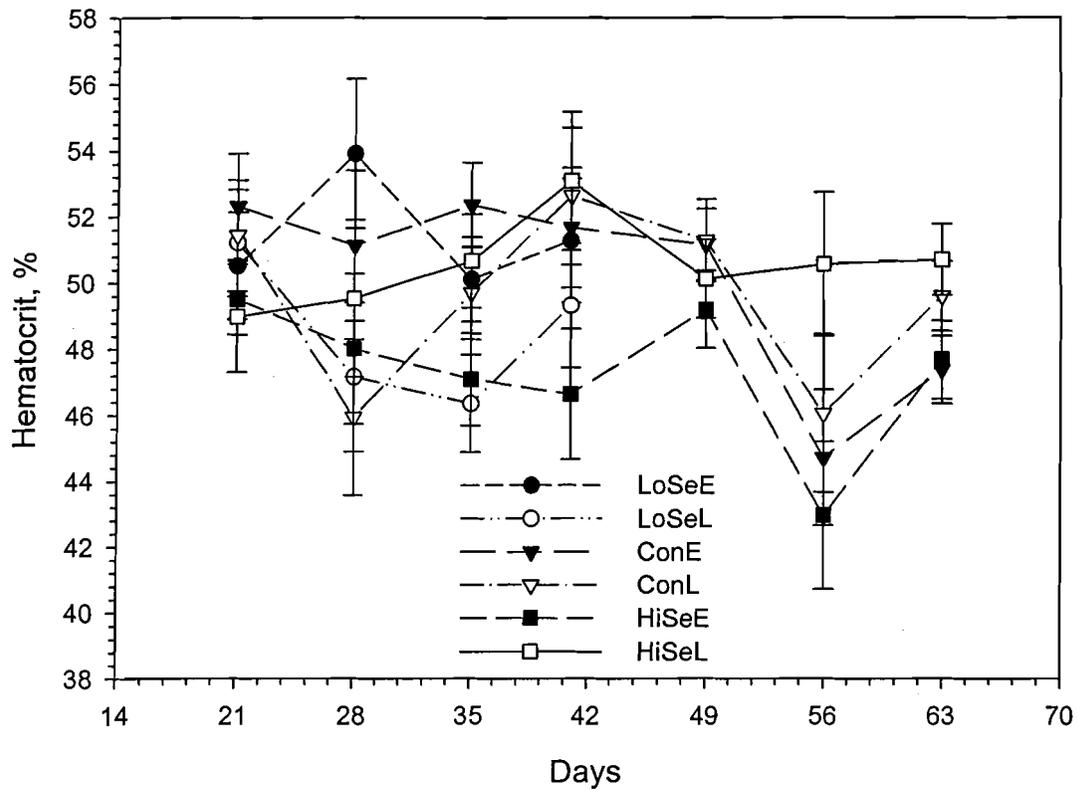


Figure 7.2.2 Hematocrit numbers in sheep (mean \pm standard error).

Normal Range: 24 -50%

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.2.2 Level of significance (< 0.05) of hematocrit numbers in sheep.

Days	Level of Significance < 0.05
	No relevant significance

creatinine. Aspartate aminotransferase levels may also be increased with liver disease (Coles, 1986; Kaneko, 1989).

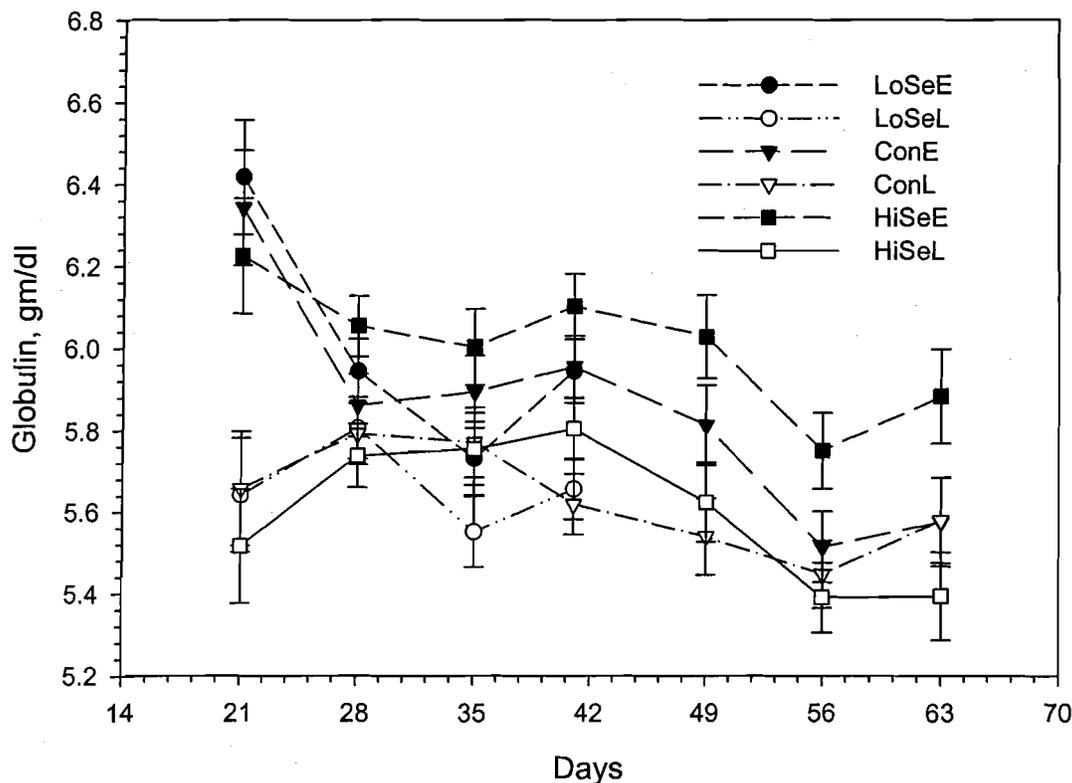


Figure 7.2.3 Serum globulin levels in sheep (mean \pm standard error).

Normal Range: 3.4 – 4.8 gm/dl

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.2.3 Level of significance (< 0.05) of serum globulin levels in sheep.

Days	Level of Significance < 0.05
	No relevant significance

7.3 Serochemistries Below the Normal Reference Ranges

Albumin is synthesized by the liver and functions as an osmotic pressure regulator, may act as the primary source of reserve amino acids for tissue proteins and plays an important role in fatty acid transportation (Coles, 1986). It is the most prominent of the serum proteins constituting between 35-50% of the total serum protein in animals. An increase in levels may indicate dehydration. A decrease may indicate liver, kidney,

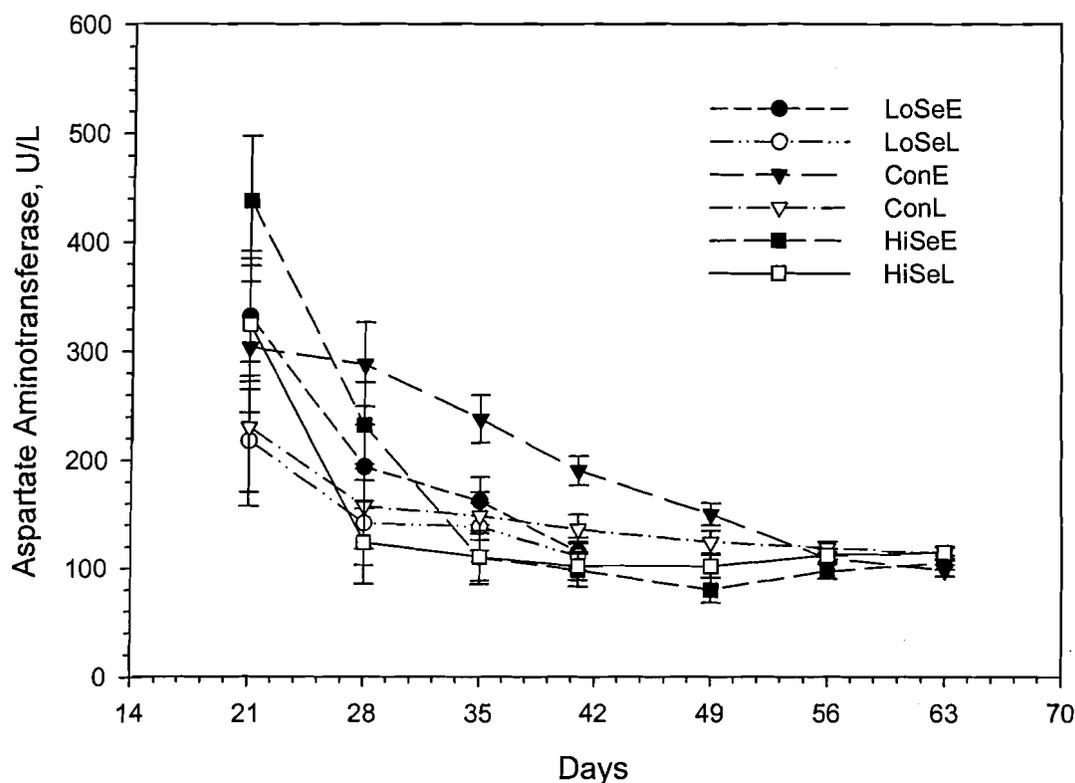


Figure 7.2.4 Serum aspartate aminotransferase levels in sheep (mean \pm standard error).
Normal Range: 81 – 143 U/L

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) –
Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.2.4 Level of significance (< 0.05) of serum aspartate aminotransferase levels in sheep. Control Ewe (CE) – High Ewe (HE) – Low Ewe (LE)

Days	Level of Significance < 0.05
35	CE – HE; CE – LE
42	CE – HE; CE – LE
56	CE – HE

gastrointestinal disease, malnutrition, blood and plasma loss (Kaneko, 1989). Other studies have reported that in sheep parasitized by *Haemonchus* organisms, there is a reported decrease in albumin concentrations and an increase in globulin concentrations (Coles, 1986).

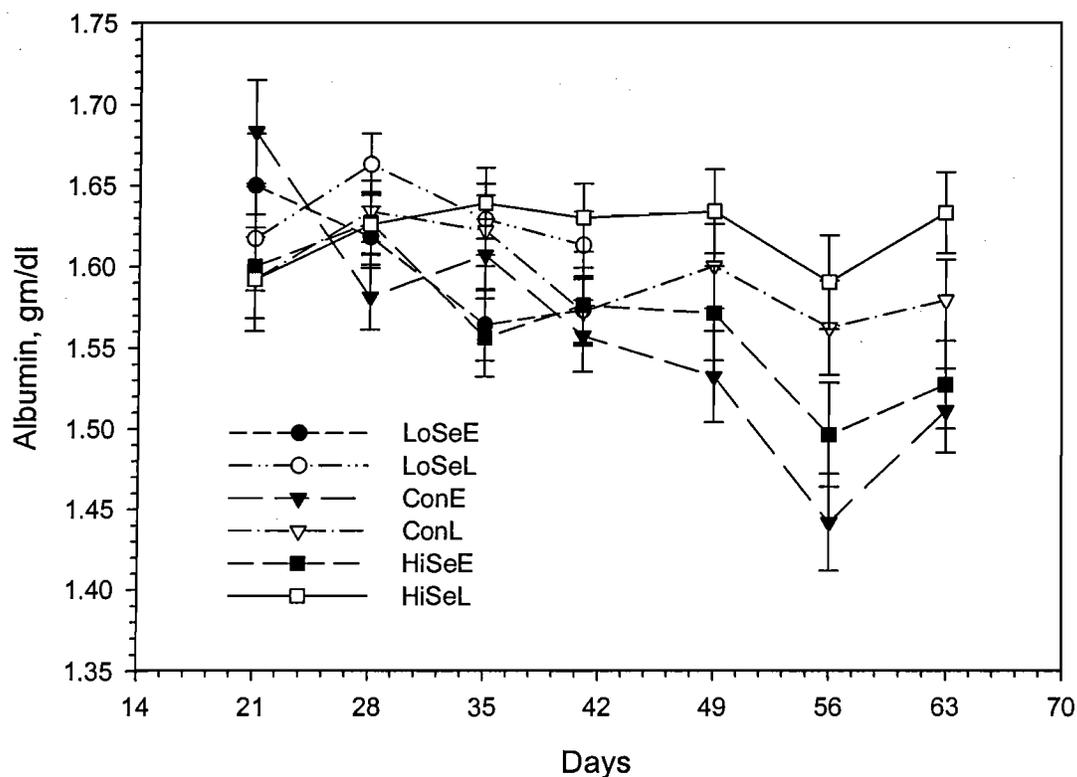


Figure 7.3.1 Serum albumin levels in sheep (mean \pm standard error).

Normal Range: 3.1 – 4.0 gm/dl

Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) – Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.3.1 Level of significance (< 0.05) of serum albumin levels in sheep.

Days	Level of Significance < 0.05
	No relevant significance

Total carbon dioxide is the second most important anionic fraction in serum. The measure of this value is important in the evaluation of acid-base disorders. A decrease in total carbon dioxide often results in metabolic acidosis. An increase in total carbon dioxide may result in alkalemia (Henry, 1996).

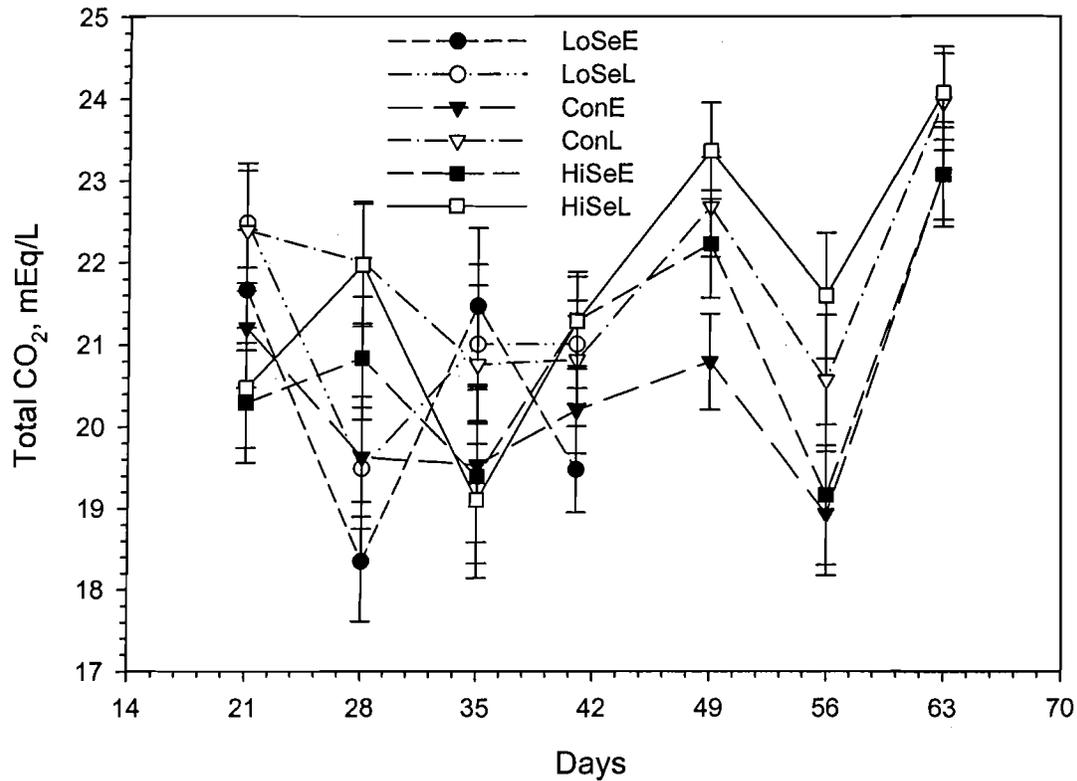


Figure 7.3.2 Total carbon dioxide (CO_2) levels in sheep (mean \pm standard error).
 Normal Range: 21 – 28 mEq/L
 Low Selenium Ewes (LoSeE) – Low Selenium Lambs (LoSeL) – Control Ewes (ConE) –
 Control Lambs (ConL) – High Selenium Ewes (HiSeE) – High Selenium Lambs (HiSeL)

Table 7.3.2 Level of significance (< 0.05) of total carbon dioxide levels in sheep.
 Control Lamb (CL) – Low Lamb (LL)

Days	Level of Significance < 0.05
28	CL - LL

8.0 Appendix

**University of Idaho
Animal Care and Use Committee**

Date: Sunday, May 12, 2002
To: Greg Moiler
From: Animal Care and Use Committee
Re: Protocol 2002-49
Selenium Toxicity and Depuration in Sheep in Reclaimed Mining Areas

Your animal care and use protocol for the project shown above was reviewed by the Animal Care and Use Committee on Sunday, May 12, 2002.

This protocol was originally submitted for review on: Monday, April 15, 2002
The original approval date for this protocol is: Sunday, May 12, 2002
This approval will remain in affect until: Monday, May 12, 2003
The protocol may be continued by annual updates until: Thursday, May 12, 2005

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.


IACUC Representative

**University of Idaho
Animal Care and Use Committee**

Date: Tuesday, July 09, 2002
To: Greg Moller
From: Animal Care and Use Committee
Re: Protocol 2002-49
Selenium Toxicity and Depuration in Sheep in Reclaimed Mining Areas

Your requested ammendment to the animal care and use protocol shown above was reviewed by the Animal Care and Use Committee on Tuesday, July 09, 2002.

This protocol was originally submitted for review on: Monday, April 15, 2002
The original approval date for this protocol is: Sunday, May 12, 2002
This approval will remain in affect until: Wednesday, July 09, 2003
The protocol may be continued by annual updates until: Thursday, May 12, 2005

Comments

Your request to euthanize and perform necropsies on the 6 animals specified has been approved.

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.


IAUCUC Representative