AN ABSTRACT OF THE THESIS OF

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Title: Effect of Selenium Source and Supplementation Rate in Ewes on Selenium Status, Passive Immunity, and Growth Performance of their Lambs.

Abstract approved:_____

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Selenium (Se) is an essential micronutrient of sheep. Supplementation is especially important in young lambs in order to prevent Se-deficiency signs. In the United States, the FDA regulates Se supplementation to ruminant diets at a level of 0.3 mg/kg Se (as fed basis). Questions still exist regarding what chemical form of Se is the most bioavailable supplement and what are the best supplementation rates for optimal productivity. Three interrelated experiments from the same study were conducted 1) to evaluate the effect of Se source and supplementation rate in ewes on Se status of their lambs at birth, 2) to examine the effect of Se source and supplementation rate on IgG concentrations in ewe colostrum and lamb serum, and 3) to evaluate the effects of Se source and supplementation rate on ewe reproductive performance and subsequent vitality and growth performance of their lambs.

For these experiments, 240 ewes were divided into 8 treatment groups and drenched weekly (at an amount equal to their summed daily intake) for one year, including during gestation and early lactation, with no Se (deficient); at recommended

levels (0.3 mg/kg) with inorganic sodium selenite, sodium selenate, or organic Se-yeast (Se-Y); or at supranutritional levels (0.9 and 1.5 mg/kg) with sodium selenite or Se yeast. In the third experiment, 88 ewes continued the study into year two for an additional 28 weeks. Year two treatments included no Se (deficient) ewes (n = 25); ewes supplemented at recommended levels (0.3 mg/kg) with organic Se-Y (n = 20); ewes supplemented at supranutritional levels (0.9 and 1.5 mg/kg) with organic Se-Y (n = 18 and 27, respectively).

In the first experiment, which assessed the effects of Se source and supplementation rate in ewes on Se status of their offspring, we found that Se administered by weekly drenching of ewes during gestation and early lactation was effective at increasing Se concentrations in ewe colostrum and milk at 30 days in milk (DIM), and in improving the Se status of lambs (whole-blood and serum Se at birth, and skeletal-muscle Se at 14 days of age) (all $P < 0.001$). Selenium concentrations in lacteal secretions and lambs were higher in ewes drenched with Se yeast compared to ewes drenched with inorganic Se sources ($P < 0.01$). Selenium concentrations in lacteal secretions and in lambs increased linearly with supranutritional concentrations of Se yeast ($P < 0.001$), whereas Se concentrations did not differ in ewes drenched with 0.9 or 1.5 mg/kg of inorganic sodium selenite ($P > 0.05$). In summary, weekly oral drenching of ewes with Se yeast during gestation and early lactation was found to be an effective method for improving Se status of their newborn lambs.

In the second experiment, which examined the effects of Se source and supplementation rate in ewes on colostral IgG and lamb serum-IgG concentrations,

we measured colostral and lamb serum-IgG concentrations at parturition, and lamb serum-IgG concentration again at 48 hours postnatal. Although Se drenching of ewes was effective at increasing whole-blood Se concentrations in ewes and lambs ($P < 0.001$), and colostral Se concentration ($P < 0.001$), there was no consistent or significant increase in IgG concentrations in ewe colostrum nor lamb serum at 48 hours of age ($P > 0.05$) irrespective of Se source, or supplementation rate. Therefore, we conclude that Se supplementation in ewes, and the Se status of newborn lambs, have little effect on IgG concentrations in colostrum and subsequent IgG concentrations in lamb serum at 48 hours postnatal.

The purpose of the third experiment was to examine the effect of Se source and supplementation rate on ewe reproductive performance and subsequent vitality and growth performance of lambs, over two consecutive lambing seasons. In year one, lambing percent; number of lambs born per ewe; lamb birth weights; 90- and 120-day BW and ADG; and pounds of lamb weaned per ewe were measured. The purpose of the second year of this study was to continue our observations for the SeY treated ewes, adding additional parameters to monitor ewe reproductive performance (percentage of ewes exhibiting estrus; percentage of ewes exhibiting estrus that lambed; percentage of ewes lambing; and number of lambs born per ewe). Year two lamb performance measures included birth weights; neonatal vigor scores; 10-, 20-, and 60-day BW and ADG; and percentage of lambs surviving to 60 days.

In year one, Se supplementation, regardless of supplementation rate, did not affect lambing percent, number of lambs born per ewe, lamb birth weights, 90-day

BW and ADG, or pounds of lamb weaned per ewe. However, lambs from ewes in the 1.5 mg/kg SeY group had greater120-day BW ($P = 0.10$) and ADG ($P = 0.06$) than lambs from ewes in the 0.3 mg/kg group. This same trend was apparent in a subgroup of lambs from Suffolk ewes receiving Se at 1.5 mg/kg that had greater 120-day BW ($P = 0.07$) and ADG ($P = 0.09$) compared to lambs receiving SeY at 0.3 mg/kg. Similarly, a subgroup of lambs reared as twins from ewes receiving Se at 1.5 mg/kg had greater120-day BW ($P = 0.07$) and ADG ($P = 0.08$) than lambs reared as twins from ewes in the 0.3 mg/kg group.

In year two, there were two ewe reproductive performance measures that were negatively affected by Se supplementation. First, the percentage of ewes exhibiting estrus that lambed in the 0.3 mg/kg SeY group (75%) was lower compared to ewes receiving no Se supplement (100%; $P = 0.02$). Also, the percentage of ewes lambing was lower ($P < 0.04$) for ewes receiving 0.3 mg/kg SeY (75%) compared to ewes receiving no Se supplemental (96%). Se supplementation, regardless of source and supplementation rate did not affect percentage of ewes exhibiting estrus, or number of lambs born per ewe. There were three lamb performance measures that were affected by Se supplementation. Lambs from ewes receiving Se at 1.5 mg/kg tended to have greater lamb vigor scores compared to lambs from ewes receiving SeY at 0.3 mg/kg ($P = 0.07$). Second, the 60-day ADG was greater for lambs from ewes in the 1.5 mg/kg group compared to lambs from ewes in the no Se group ($P = 0.07$). A subgroup of lambs from Suffolk ewes receiving Se at 1.5 mg/kg had greater 60-day BW ($P = 0.03$) and ADG ($P = 0.06$) compared to lambs receiving SeY at 0.3 mg/kg. Similarly,

a subgroup of lambs reared as twins from ewes receiving Se at 1.5 mg/kg had greater 60-day BW ($P = 0.08$) and ADG ($P = 0.06$) than lambs reared as twins from ewes in the 0.3 mg/kg group. Third, there was an increased percentage of lambs surviving to 60 days in lambs from ewes in the 1.5 mg/kg group compared to lambs from ewes in the no Se group (86% vs. 64%, respectively; $P = 0.04$). Se supplementation, regardless of source and supplementation rate, did not affect lamb birth weight nor 10- and 20-day BW and ADG.

We conclude that supranutritional Se supplementation of ewes, primarily with SeY, positively affects later lamb performance measures (i.e., 60- and 120-day BW and ADG), and that these effects may be more pronounced for lambs of heavier breed (i.e., Suffolk) and lambs reared as twins.

Effect of Selenium Source and Supplementation Rate in Ewes on Selenium Status,
Passive Immunity, and Growth Performance of their Lambs.

by

Whitney C. Stewart

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Whitney C. Stewart, Author

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CHAPTER 1. INTRODUCTION

Selenium (Se) is well known as an essential trace mineral in sheep and cattle. Selenium was discovered as an essential micronutrient in 1958. Much of the ground work for the discovery of Se was done here at Oregon State University through the efforts of Dr. James Oldfield and others. After its discovery, researchers focused initially on the quantity of dietary Se needed to prevent Se-responsive diseases, such as nutritional myodegeneration and selenium responsive unthriftiness (SeRU) (Muth et al., 1958). More recently, researchers have shifted their focus to the source and supplementation rate of Se needed to maximize biological functions. Questions still exist regarding what chemical form of Se [e.g., sodium selenite, sodium selenate, or selenium yeast (SeY)] is the most bioavailable supplement; and whether the FDA recommended 0.3 mg/kg concentration is sufficient to satisfy requirements in sheep.

My involvement with Se research here at OSU began when I was provided the opportunity to assist Dr. Jean Hall and Gene Pirelli as a graduate research assistant initially studying the effects of Se on foot rot in sheep. Apart from my responsibilities in helping with their study, I had to determine what aspects of Se research I would focus on for my graduate research. Given my background in livestock production, my approach initially was focused on the effects of Se source and supplementation rate on production parameters. However, with the guidance of my graduate committee and

as a result of my course work, my research evolved into determining the effects of maternal Se supplementation on 1) how Se is transferred to the newborn lamb, 2) its effects on passively acquired immunity in lambs, and 3) ewe reproductive performance and lamb growth and performance in the periods that follow parturition. Thus, the findings contained in this thesis are a small part of the larger USDA funded project, and are the result of hard work and dedication by all those involved with the collection, processing, analysis, and presentation of data from this study.

CHAPTER 2.   REVIEW OF LITERATURE

2.1   SELENIUM BIOAVAILABILITY

2.1.1   Selenium Sources

Selenium (Se) was discovered as an essential micro-nutrient in 1958. After its discovery, research was initially focused on the quantity of dietary Se that needed to be consumed to prevent Se-deficiency related diseases. More recently, research has focused on the best sources of Se to maximize biological functions.

The element Se belongs to group VI in the periodic table of elements. Its atomic number is 34 and atomic weight is 78.96. In nature Se exists in two forms: inorganic and organic Se. From Elemental Se (Se^0), inorganic Se can be reduced to selenide (Se^{-2}) or oxidized to selenite (Se^{+4}) or selenate (Se^{+6}). Of the four forms of Se that exist in nature, only selenite and selenate are available for uptake by plants and incorporation into organic forms of Se (e.g., selenomethionine and selenocysteine) (Surai, 2006). The most abundant sources of inorganic Se used for dietary supplements are the Se salts: sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4).

Selenium sources in the form of selenoamino acids are considered organic Se. Although organic Se originates from selenite or selenate in the soil, in the plant they are transformed and incorporated into Se-containing proteins. Selenium yeast (SeY) is the most common organic Se supplement whereas corn, wheat, soybeans, fishmeal, and any feedstuff high in Se content can also be considered an organic Se source. The transformation of Se from inorganic to organic forms results from its incorporation

into amino acids. Selenium is chemically similar to sulfer (S). Both form integral molecules of the amino acids methionine (Met) and cysteine (Cys). In fact, the interchangeability of Se for S in methionine and cysteine results in formation of the selenoproteins selenocysteine (SeCys) and selenomethionine (SeMet) (Schrauzer, 2000; Surai, 2006). Similar to Met, Seemed is not synthesized by mammals, and therefore, must come from feed sources. Selenomethionine represents over 50% of Se in cereal grains and greater than 80% of Se in SeY. Other selenocompounds present in plant species include Se-methyl-selenomethionine and Se-methyl-selenocysteine (Whanger, 2002).

The bioavailability of a trace mineral refers to the degree to which an ingested nutrient is absorbed and available to the body. The chemical form of Se exerts the greatest influence on its bioavailability. However, other factors also contribute to the bioavailability of Se including other dietary components, the degree of protein digestion, and the stage of physiological development (Surai, 2006).

The inorganic Se supplements sodium selenite and sodium selenate are thought to have similar bioavailability, whereas organic Se supplements such as SeY may have greater bioavailability because they effectively raise Se concentrations in blood and milk of ruminants. Podoll et al. (1996) examined differences in bioavailability between sodium selenite and sodium selenate in sheep, cattle, and horses. No significant differences were observed in serum Se concentrations, glutathione peroxidase activity (GSHPx), skeletal muscle Se concentrations, or liver Se concentrations when comparing sodium selenite and sodium selenate.

When comparing the bioavailability of inorganic Se to SeY, numerous studies have found greater fetal transferability and digestive absorption with SeY. Approximately 66% of SeY ingested is absorbed as opposed to approximately 50% of sodium selenite (Weiss, 2005; Vignola et al., 2005). An overall enhanced absorption of organic Se sources in sheep has been observed in studies measuring Se concentrations in blood, muscle, and milk (Abd El-Ghany et al., 2007, 2008; Dominguez Vara et al., 2009; Rock et al., 2001; Taylor et al., 2009). Ortman & Pehrson (1999) found that SeY was much more effective at raising Se concentrations of milk than inorganic Se supplements. Increased Se concentrations in milk and colostrum with SeY results from greater amounts of methionine in milk proteins than whole-blood proteins (Weiss, 2005).

The incorporation of SeMet into animal proteins allows greater Se storage and retention with SeY than Se from inorganic sources. Juniper et al. (2008) found that lambs supplemented with SeY at 0.18 mg/kg of diet for 90 days maintained adequate Se status for greater than 300 days. Thus, adequate Se status of the animal is achieved with a shorter supplementation period using SeY.

The mere ingestion of a Se containing mineral does not ensure that the mineral is available for use in the body. If not present in an available chemical form, then the supplement is excreted and the animal's requirements are not met. The efficiency of Se supplementation begins and ends with the bioavailability of the chemical form of Se.

2.1.2   Selenium in Plants and Soils

Selenium-deficient soils are those that have less than 0.5 mg Se/kg soil (NRC, 2007). Soil with greater than 1.0 mg Se/kg soil is considered seleniferous, meaning it has sufficient to excess Se concentrations (Mayland et al., 1989). Selenium-deficient soils in the Pacific Northwest were likely caused by volcanic activity whereby the Se was lost as volatile gases because of high temperatures. Selenium-deficient soils of the Northeast and Great Lake regions are derived from sedimentary rocks of the pre-cretaceous periods (300 to 145 million years old). The Southeastern states are also deficient in Se, because those soils were derived from highly weathered coastal deposits. Shale-derived soils, especially those developed during the cretaceous period (145 to 60 million years ago) have high soil Se concentrations and are found in the states of SD, MT, WY, NE, KS, UT, CO, and NM. Such soils can contain 1 to 300 mg Se/kg soil. Internationally, Se-deficient regions include Latin America, Northern Europe, New Zealand, Australia, Indonesia, Kenya, the Philippines, and China. It is important to note that even within Se-deficient regions, pockets of seleniferous soils can exist. Soils can range from Se-deficient to Se-toxic within 20 km of each other (Fordyce et al., 2000). Knowing the underlying geo-physical characteristics of a soil site will be more helpful in predicting the Se status of a soil than more generalized Se maps.

Unfortunately, soil-Se content and plant-Se uptake are not always closely correlated. Therefore, Se deficiency in animals is not always caused by low Se soil-content. In many instances, the chemical forms of Se in the soil are not available to

the plants. Climatic factors also have a profound influence on what chemical forms of Se are present in the soil. With poorly drained soils, reducing conditions generate selenide (Se^{-2}) and elemental Se (Se^{0}), which are unavailable for plant uptake (Hawkesford et al., 2007). This is seen in regions of high precipitation. Selenite is available for plant uptake in soils with a pH less than 7, but is less available for plant uptake than in alkaline soils.

Generally Se is available for plant uptake in areas where the soil was initially high in Se and developed under arid conditions. Selenate is the predominant source of Se for plant uptake in alkaline soils with a pH greater than 7. The adsorption tendency of selenite, i.e., its attraction to other molecules in the soil, affects plant uptake of selenite from the soil. Selenite is strongly bound to the oxides of Fe and Al and less mobile in the soil, whereas selenate is weakly adsorbed and moves more readily through the soil. Consequently, plant uptake of selenate is greater than selenite, yet the mobility of selenate results in greater leaching with high rainfall and irrigation (Hawkesford et al., 2007). Selenium bioavailability to plants is decreased by high clay content, decreasing pH, and excessive organic matter accumulation. Crop uptake of Se was also observed to decrease with compaction of the soil (Zhao et al., 2007).

Whereas Se is essential for animal health and nutrition, it is not considered essential for plant growth. Plant uptake of Se from the soil occurs via a sulfate transporter in the plant's roots. Both selenite and selenate are available to the plant for uptake under the right soil conditions, yet translocation throughout the plant is more limited with selenite compared to selenate (Hopper and Parker, 1999). Studies

comparing plant availability of these two inorganic forms have shown that selenate has a two-fold faster plant uptake and fewer losses of Se to volatilization when compared to selenite (de Souza et al., 1998). Thus, Se as selenate remains the more efficient source of Se for plants, in uptake, mobility and persistence within the plant (Whanger, 2002). An antagonistic relationship occurs in instances where excessive amounts of S and Se compete for absorption at the root. Plants cannot distinguish between these two elements because of their similar chemical properties. This is especially important when using sulfate fertilizers, which negatively affect Se absorption. Once absorbed by the plant, selenate like sulfate is transported around the plant and accumulates in mature portions of the plant. This is especially common in the Se-accumulating plant *Astragulus bisulcatus* whereby selenate accumulates in older leaves. Similarly, wheat will re-mobilize Se into the grain kernels at the initiation of senescence, but in the form of SeMet (Hawkesford et al., 2007).

It is important to note that not all plant species can take up Se to the same extent. Selenium-accumulator plant species are generally found in areas with high soil-Se content and they absorb high amounts of Se (50 to 1000 mg Se/kg DM of plant). Plant species such as *Astragalus, Machaeranthera, Haplopappus*, and *Stanleya* are classified as Se-accumulators. In contrast, common forages grown on seleniferous soils accumulate low levels of Se. Species such as white clover, buffalo grass, and grama grass are poor accumulators of Se. In soils with low levels of Se, Massey & Martin (1975) found that alfalfa accumulates more Se than red clover, timothy, or brome grass. Se uptake by plants in soils with low Se status might be

attributed to the depth of the root system. Deeply rooted, well established forage species may reach Se species in soil better than shallow-rooted forage stands. Whereas alfalfa might be considered the best Se-accumulating forage in marginally Se-deficient soils, wheat is the best cereal grain Se-accumulator species.

The difference between Se-accumulator and non Se-accumulator plants is the Se-accumulator species' ability to convert seleno-proteins into amino-acid derivatives using the enzyme Se-methyltransferase (SMT) (Hawkesford et al., 2007). Hence, Se-accumulator plant species tolerate excessively high Se concentrations by further converting SeCys to its amino-acid derivative Se-methyl-selenocysteine (MeSeCys) (Hawkesford et al., 2007). Additional oxidation and methylation steps result in the production of Se-containing volatiles, which can then be eliminated from the plant. Research efforts are underway to try and incorporate the Se-accumulating Astragalus gene for SMT into non Se-accumulating plant species in hopes of increasing the plant's ability to take up and store Se (LeDuc et al., 2004).

Researchers in locations where human and animal populations suffer from Se deficiency are particularly interested in breeding and selecting for plant Se absorption, e.g., in cereal grains as a means of fortifying food supplies. Methods of increasing soil Se concentrations with selenate fertilizer provide a short term solution to combating Se-deficiency related problems. Filley et al. (2007) determined that Se fertilization of soil was effective at meeting ruminant requirements, but only for two consecutive years following fertilization. Biannual Se-fertilization of crops can be labor intensive. Plant breeding and selection for traits such as Se accumulation and/or

the ability to re-mobilize Se to edible plant portions might be a more sustainable approach, especially for human nutrition. Unfortunately, Se fortification of plants, whether for human or animal consumption, is ultimately limited by Se availability in the soil.

2.1.3 Selenium Metabolic Pathways

More than 30 seleno-proteins have been identified in various mammalian tissues, although only 20 have known biological functions. These seleno-proteins are further categorized into three groups according to their involvement in cellular functions: 1) the glutathione peroxidases (GSHPx) responsible for reducing hydroperoxides in cells, 2) the thioredoxin reductases involved in cell growth and apoptosis, and 3) the iodothyronine deiodinases involved in thyroid hormone metabolism. Interestingly, with seleno-proteins distributed throughout many tissues in the body, certain tissues will retain Se or become Se-depleted more rapidly than others. Selenium in skeletal muscles is depleted more rapidly than Se in the brain, endocrine, and reproductive organs, which explains the most common clinical signs associated with Se-deficiency (Abd El-Ghany et al., 2010).

When inorganic Se sources are consumed, i.e., dietary sodium selenite or sodium selenate, they must first be reduced to the central Se metabolite hydrogen selenide (H_2Se) (Figure 1). Hydrogen selenide is then converted to selenophosphate ($HSePO_3^{2-}$) and incorporated by specific UGA codons into selenoproteins. If present in excessive amounts, H_2Se is oxidized by a cascade of reactive-oxygen species. Superoxides are produced and lead to toxicosis. Excess Se can also be excreted when

Se is transformed into methylated metabolites such as methyl selenol (CH_3SeH), which is excreted in urine as $(CH_3)_3Se^+$, or dimethyl selenide $(CH_3)_2Se$, which is exhaled in breath.

In contrast, when organic Se sources are consumed, Se is non-specifically incorporated into tissue proteins. For example, SeMet is incorporated into proteins in place of methionine. The SeMet can also be catabolized from proteins and then trans-selenated to SeCys, which is then converted to H_2Se by a ß-lyase. The H_2Se can then be converted to $HSePO_3^{2-}$, thus following the path of specific incorporation into selenoproteins. Selenium from organic sources is also excreted in the urine or exhaled by the lungs in similar fashion to Se consumed as selenite or selenate (Rayman et al., 2008).

The differences between specific versus non-specific incorporation of Se can be summed up by understanding that the specific selenoprotein synthesis pathways of inorganic Se sources are "homeostatically" controlled and cannot be increased by additional Se supplementation, whereas the non-specific pathways that incorporate SeMet into tissue proteins can be increased because the tRNA does not distinguish between methionine and selenomethionine (Behne et al., 2001). In situations whereby dietary methionine is limited, SeMet is preferentially incorporated into body proteins.

Figure.1. Metabolic pathways of dietary Se (adapted from Raymen et al., 2008)
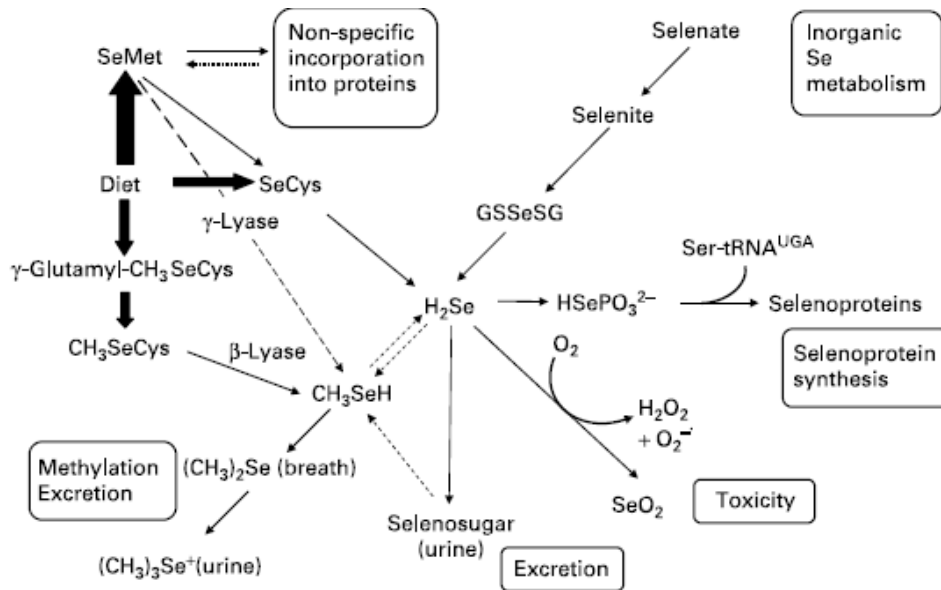
Ruminants metabolize Se differently than monogastric animals (Abd El-Ghany et al., 2010). The main site of Se absorption is the duodenum, although there is slight absorption in the abomasum. The percentage of inorganic Se absorbed in the duodenum from an oral dose is 77 to 85% in non-ruminants, whereas in ruminants only 11 to 35% of Se given orally is absorbed in the duodenum (Abd El-Ghany et al., 2010; Spears, 2003). One explanation for the lack of absorption in ruminants is attributed to the reductive environment of the rumen, in which inorganic Se is reduced to insoluble forms such as Se^0 and H_2Se (Abd El-Ghany et al., 2010; Surai, 2006). The Se that is not reduced to insoluble forms is incorporated into rumen microorganisms. Peter et al. (1992) discovered that significantly more Se from selenite was converted to insoluble forms by rumen bacteria than Se from SeMet. Van Ryssen (1998) found that rumen bacteria were sensitive to changes in Se intake. This is in agreement with Whanger (2001) who showed that Se concentrations in rumen flora were 46- times greater than in the consumed diet. Selenium metabolism by rumen bacteria is highly dependant on the bacterial species present. Pure cultures of *Selenomas ruminantium* and *Butyrivibrio fibrisolvens* incorporated Se into seleno-aminoacids in contrast to *Bacteroides ruminicola* whereby only elemental Se was formed (Hudmann and Glenn, 1984). Selenium incorporated into rumen microorganism as elemental Se is thought to have low digestive bioavailability in the duodenum (Surai, 2006). Serra et al. (1997) found that Se in rumen bacteria collected from sheep and fed to rats had low digestive bioavailability, whereby only 25% of the

total Se present in the diet was absorbed by the rats, thus indicating that in many instances certain rumen microorganisms reduced Se bioavailability.

Rumen fermentation studies have shown that the effects of Se on rumen fermentation are highly dependant on the Se source used and dietary components. Kim et al. (1997) found that Se as SeMet resulted in greater short chain fatty acid production by rumen microorganisms when compared with sodium selenite. Koenig et al. (1997) discovered that Se bioavailability in ruminants was also influenced by the composition of the animal's diet, with greater absorption when diets were high in concentrates and lower absorption when diets were forage based. This supports the notion that Se absorption in the rumen is in part dependent on microbial populations (e.g., bacteria, fungi, and protozoa) and the various ways each will metabolize Se. A dramatic difference in rumen microbe populations exists between a diet high in concentrates versus a diet high in forages. Hudmann and Glenn (1984) showed that the rumen bacteria who most efficiently metabolized Se into seleno-proteins (*Selenomas ruminantium* and *Butyrivibrio fibrisolvens*) are the same starch digesting bacteria most commonly found in rumens of animals consuming high amounts of concentrates. In contrast, greater populations of protozoa, fungi, and cellulolitic bacteria are found in rumens of animals consuming forages, which explains the decreased Se absorption in animals fed diets high in forage (Tajima et al., 2000).

An interaction of Se with other dietary components also plays an important role in Se absorption in ruminants. The sulfur content of the diet is of major importance, although vitamins and minerals also have been shown to interfere with

Se uptake. An antagonistic relationship exists between S and Se because of their similarities in chemical and physical properties. High concentrations of dietary S will decrease biological availability of Se in sheep. Muth et al. (1961) reported that the addition of S as sodium sulfate to the diet decreased the effectiveness of dietary sodium selenite in preventing nutritional myopathy.

In a study looking at calcium's interaction with Se, Harrison and Conrad (1984) found that Se absorption in non-lactating dairy cows was optimal when cows were supplemented with 8 g of calcium/kg of diet. A three-way antagonistic interaction involving copper, S, and Se has been proposed. For example, van Ryssen and Hatmann (1997) measured a decrease in rumen microbial Se concentration and muscle tissue-Se concentration when copper was added to the diet. Copper exerts little to no effect on Se absorption, but increasing concentration of S decreased both Se and copper concentrations in the liver of sheep. It is clear that S exerts the most profound effect on Se absorption (van Ryssen and Hatmann, 1998; Spears, 2003).

Selenium bioavailability is also affected by the cyanogenic glycoside content of forage or concentrates (Spears, 2003). Certain legumes, e.g., white clover, can be high in cyanogenic glycosides, which antagonize Se absorption in the rumen. Gutzwiller (1993) fed a variety of white clover high in cyanogenic glycosides and observed a decrease in whole-blood Se concentrations when compared to ewes grazing a white-clover variety low in cyanogenic glycosides (Spears, 2003).

Inorganic selenite reacts with ascorbic acid (vitamin C) causing Se to be reduced to the unavailable Se^0 form. Diets containing 0.25% vitamin C impeded the

uptake of Se in the diet when fed at 0.3 mg/kg, by reducing the sodium selenite to Se^0 (Robinson et al., 1985). Not only was Se lost, but also the ascorbic acid was oxidized thereby losing its biological activity as well (Surai, 2006). This can happen before a mineral premix leaves the bag and is noticeable by the formation of pink particles in the mixture (Surai, 2006). The tendency for selenite to be reduced to elemental Se^0 while in the bag was correlated to increased Se excretion in pigs fed stored Se premixes versus freshly prepared premixes (Groce et al., 1973). The possibility of Se interacting with other premix ingredients exists. The use of more stable organic Se sources such as SeY would increase the bioavailability of Se in mineral pre-mixes.

2.1.4   Selenium Supplementation Strategies

Ideally, adequate Se is provided to the animal via forage sources that contain Se in the form of SeMet or SeCys. Unfortunately, many soils in the U.S. are Se deficient, i.e., in the Northeast, Northwest, Southeast and Great Lake regions, thereby necessitating Se supplements are provided for animals grazed in these regions. Methods of supplementation include free-choice Se-mineral supplements, injections of Se, Se in oral drenches, Se soil amendments, and Se rumen boluses. In Se-deficient regions the two most common methods of Se supplementation include providing a free choice Se-containing mineral mixture or using an injectable form of Se (sodium selenite with Vitamin E). Inherent challenges exist with current supplementation strategies.

Dargatz and Ross (1996) demonstrated the challenges of meeting Se requirements with a mineral premix in a survey of 253 cow-calf operations

throughout the U.S. They found that even in operations that provided a Se-supplementation program, greater than 30% of the animals in the herd were still deficient to marginally deficient. The inconsistent Se status of animals within the same herd under the same management conditions suggests that provision of Se-fortified premixes does not guarantee that all animals are consuming and absorbing sufficient Se. Ducker et al. (1981) evaluated individual intake of mineral supplement in 2,900 grazing ewes from 15 different flocks and found that 19% of ewes did not consume any block and 36% of ewes were classified as low consumers.

There are likely several reasons for such varied mineral-mix consumption, including mineral location, palatability issues associated with weather damage, and unfamiliarity with feeding apparatuses (Bowman and Sowell, 1997). Ruminants grazing on large parcels are unlikely to find and consume mineral mixes on a consistent basis. Time, labor and economic resources limit how consistently Se is provided, making free choice supplementation problematic. Fluctuations in individual consumption, human error, and limited resources are only part of the challenge in meeting Se requirements. Differences in bioavailability of the various Se sources and in the methods of administration both determine the success of a supplementation strategy.

Direct subcutaneous injections of sodium selenite at 10 to 30 mg (cattle) and 1 to 5 mg (sheep) are effective in preventing Se-responsive diseases, but are costly ($0.36 for 30 days) and of short duration, with a 45-day average (McDowell et al., 2002). McDowell et al. (2002) conducted a 24-month comprehensive study

comparing 1) a control (no Se supplementation), 2) 5-ml of injectable sodium selenite every six months, 3) 9-ml of barium selenite given once at trial initiation, and 4) SeY in mineral mixture at 2.1 mg per cow per day. For all time points, the SeY mineral mixture was superior at increasing Se concentrations in plasma of grazing beef cattle, with barium selenate being the second most effective. The groups receiving no Se and sodium selenite injections showed no difference in plasma-Se concentrations.

Injectable barium selenate has been shown to maintain Se concentrations for 200 weeks in ewes and lambs although barium selenate is not readily available in the U.S. Whereas Se injections may be beneficial in production systems where animals can be handled at birth and re-injected every 30 days, it is unlikely that time and labor resources are available for this on many livestock operations.

2.1.5 Selenium Recommendations and Tolerances

FDA regulations and NRC recommendations dictate how much Se can be included in mineral supplements. Despite the discovery in 1957 that Se protects sheep against nutritional myopathy, it wasn't until 1978 that Se was approved at 0.1 mg/kg as a feed supplement for ewes and lambs (FDA, 1978). In 1987, recommendations increased from 0.1 mg/kg to 0.3 mg/kg (FDA, 1987). Current FDA regulations limit the amount of Se that can be added to complete feeds to 0.3 mg/kg or no more than 0.7 mg per head per day. Free-choice mineral supplements are allowed to contain up to 90 mg/kg; again intake is not to exceed 0.7 mg/d consumption. Dietary levels that exceed 0.3 mg/kg are referred to as supranutritional levels (NRC, 2007).

To determine Se requirements for optimal health in sheep, estimates of dietary Se bioavailability are needed. Net Se requirements for sheep are estimated using factorial models with 0.25 μg Se/kg BW for maintenance and additional coefficients accounting for Se in growing tissue, milk, fetus, and wool (NRC, 2007). The net requirement is then divided by an absorption coefficient to generate a daily requirement. Defined absorption coefficients for forages and concentrates are 0.3 and 0.6, respectively (NRC, 2007). The calculated Se dose must comply with FDA (2009) regulations, which allow ruminant diets to be supplemented with up to 0.3 mg/kg from selenite, selenate, or Se-yeast (FDA, 2005) to a maximum intake of 0.7 mg Se/d. Even with more defined Se NRC recommendations, some studies still suggest that current Se recommendations fail to meet Se requirements during periods of increased production (Davis et al., 2006; Schrauzer, 2000).

The maximum tolerance concentration (MTC) for Se is 5.0 mg/kg DM. This was increased from 2.0 mg/kg DM in 1980 (NRC, 2007), although recent findings suggest that sheep can tolerate higher levels than recommended (Christaldi et al., 2005; Davis et al., 2006; Taylor et al., 2009). Davis et al. (2006) found that feeding 12 mg/kg of selenite during gestation and lactation did not induce toxicoses. Furthermore, lambs born from ewes supplemented at 0.2, 4, 8, 12, 16 and 20 mg/kg did not show any deformities, abnormalities, or any signs of Se toxicoses from birth to weaning (Davis et al., 2006). Cristaldi et al. (2005) also found that wethers receiving 10 mg/kg selenite for 1 year did not display any signs of Se toxicoses. The MTC is also greater for organic-Se sources in the form of Se-enriched grain. For

example, Taylor et al. (2009) discovered pregnant ewe lambs were capable of consuming greater than twice the current 5.0 mg/kg MTC in the form of Se-enriched wheat grain.

2.1.6   Determining Selenium Status in Ruminants

The Se status of ruminants is commonly assessed by measuring Se concentrations in serum, whole blood, and liver; by measuring GSH-Px activity in erythrocytes and liver; and by measuring mRNA levels of GSH-Px (Kincaid, 2001). Additional tests of Se status can also be assessed e.g., by measuring Se concentrations in muscle, milk, and colostrum; and by measuring selenoprotein P concentration and deiodinase activity in tissues (Surai, 2006). Each method appears to have advantages and disadvantages. For example, serum-Se concentrations can be falsely elevated by hemolysis of erythrocytes. For this reason, whole-blood Se is considered a preferential test by some. Whole-blood Se concentrations reflect the long-term Se status of an animal because Se is incorporated into red blood cells at the time of their formation and the life span of the red blood cell is 90 to120 days. Therefore, Se content of erythrocytes reflects Se intake 1 to 3 months previously (Surai, 2006; Thompson et al., 1980). In contrast, some would argue that Se concentration in serum and plasma is advantageous because it reflects the short-term Se status or Se intake in the immediate prior weeks (Smith, 1998). Generally the ratio of whole-blood Se to serum Se is 2:1 to 4:1 (Kincaid, 2001).

Clinical deficiencies in ruminants are associated with values in whole blood less than 30 ng of Se/mL (Kincaid, 2001). Cattle with whole-blood Se concentrations

of less than 60 ng/mL are considered severely deficient, 60 to 200 ng/mL marginally

deficient, 210 to 1200 ng/mL adequate, and >1200 ng/mL as highly adequate

(Kincaid, 2001). The whole-blood Se reference range for sheep is 150 to 500 ng/mL

(Hall et al., 2009). Stowe and Herdt (1992) found acceptable serum Se-concentrations

to be 50 to 80 ng/mL for lambs and 120 to 150 ng/mL for adult sheep. Davis et al.

(2006) attained whole-blood Se concentrations in ewes in excess of 410 ng/mL when

supplemented at 0.2 mg/kg with sodium selenite for 72 weeks, and achieved levels of

1000 to1800 ng/mL when supplemented with 8 to 20 mg/kg sodium selenite for 72

weeks. Selenium reference ranges have been shown to differ, especially at the range

between optimal and suboptimal (Surai, 2006).

According to Stowe and Herdt (1992) Se concentrations may vary between

laboratories by greater than 30%. For example, GSH-Px activity is a common test for

assessing Se status, but is highly variable between laboratories as a result of

differences in methodologies, sample storage, and sample transportation (Surai,

2006). Lack of uniformity in laboratory protocols and differences in analytical

instrumentation used to measure Se concentrations both contribute to variations in Se

content of animal samples. Numerous methods have been developed for analyzing Se

concentrations of blood and tissue samples, although sensitivity and accuracy have

improved after the first analysis was reported in the 1960's. Until mid 2001, the

preferred methods for measuring Se concentrations in blood and animal tissues were

graphite furnace atomic absorption spectrophotometry (GFAAS), hydride-generation

atomic absorption spectrophotmetry (HGAAS), and molecular fluorescence

spectrophotometry (MFS). A survey in 1996 of the most common methods for Se analysis in 33 laboratories found 88% used GFAAS. Only 6% used MFS and HGAAS (Sheehan & Halls, 1999).

More recently, the method of inductively coupled argon plasma emission spectroscopy (ICP-MS) has been shown to display the highest level of sensitivity and accuracy for samples containing very low Se concentrations (Labat et al., 2001). Especially when quantifying Se concentrations in water and soils, ICP-MS has shown greater accuracy than the atomic absorption spectrophotometry techniques (Labat et al., 2001).

2.2 THE IMPORTANCE OF SELENIUM IN RUMINANTS

2.2.1 Selenium Responsive Diseases-Consequences of Deficiency

The 1958 discovery of selenium's role in preventing nutritional myopathy, a degenerative muscle disease in sheep (Muth et al., 1958) led the way to a better understanding of the multi-faceted role of Se in ruminant health and nutrition. Nutritional myopathy commonly known as "white muscle disease" is the most understood Se-responsive disease. White muscle disease merits its name because of calcium deposits that give a whitish appearance within the muscle (Hansen et al., 1993; Surai, 2006). The disease targets mainly the skeletal and cardiac muscles of new-born ruminants, although it can also affect mature ewes and rams. Increased activity of enzymes (e.g., lactate dehydrogenase, creatine phosphokinase, and pyruvic transaminase) causes muscle-tissue damage (e.g., lipid peroxidation of muscle membranes) and is responsible for tissue damage (Surai, 2006). Clinical signs are

stiffness, swollen muscles, and an inability to stand. Slight muscle tremors ranging to complete paralysis of the hind legs are also observed.

Nutritional myopathy can be classified into two forms: congenital and juvenile. The congenital form is associated with abortion, stillbirth, or death shortly after parturition. The juvenile form is observed in calves and lambs 3 to 8 weeks of age (Andrews et al., 1968).

In addition to nutritional myopathy, Se deficiency also results in decreased feed efficiency, decreased milk production, and decreased survival. In the livestock industry, even a slight decrease in overall animal health and/or reproductive performance can dramatically impact profitability. Less apparent sub-clinical signs of Se deficiency include decreased conception rates, irregular estrus cycles, longer calving intervals, and abortions. Se deficiency-related reproductive dysfunctions also manifest as retained placenta, metritis and mastitis. Studies in the dairy industry have found that Se supplementation decreases the incidence of retained placenta and metritis by up to 34% in dairy operations (Allison and Laven, 2000; Surai, 2006).

A comprehensive review by Harmon (2002) of mastitis in the dairy industry showed that supplementation with the combination of Se and Vitamin E led to a 57% reduction in mastitis in early lactation and a 32% reduction throughout lactation, with an overall 40 to 50% reduction in duration of mastitis. Smith et al. (1988) suggested that resistance to mastitis is higher in dairy cows if whole-blood Se concentrations are greater than 200 ng/mL.

In the sheep industry, losses in reproductive performance from Se deficiency are not as well quantified as in the dairy industry (e.g., mastitis, metritis, and retained placentas), although Se-related reproductive losses are observed. Gabryszuk and Klewiec (2002) showed a 32% increase in lambing percentages in marginally Se-deficient ewes injected with sodium selenite compared to controls. Sheppard et al. (1984) found Se-deficient ewes had fewer twins than ewes with adequate Se status. Bin Talib et al. (2009) showed that rams receiving Se and vitamin E had greater sperm quality (e.g., increased motility, increased sperm volume, and fewer dead and abnormal spermatozoa). Additionally, hot-weather breeding performance was enhanced with Se supplementation.

2.2.2   Selenium in Growth and Performance

Newborn lamb vitality is enhanced with Se supplementation of Se-deficient ewes. Munoz et al. (2008) found that Se-deficient ewes supplemented throughout pregnancy, compared with Se-deficient ewes supplemented only in the third trimester of gestation, had increased lamb viability and survival. The lambs from Se-supplemented ewes were quicker to stand and nurse than lambs from non-supplemented ewes (Munoz et al., 2008).

Another consequence of Se deficiency is Se-responsive "unthriftiness" (SeRU), which is characterized by poor growth rates in lambs (Grace et al., 2002). Sheppard et al. (1984) showed that lambs with whole-blood Se concentrations below 10 ng/mL had more severe cases of SeRU, whereas those lambs with Se in the range of 10 to 20 ng/mL had less severe growth depression. Numerous studies have shown

that perinatal and direct Se supplementation to lambs can enhance growth rates in

sheep (Kumar et al., 2009; McDonald, 1975; Sheppard et al., 1984). As much as a 2

kg difference in lambs at weaning has been observed as a result of Se

supplementation when compared to lambs not supplemented with Se (Grace et al.,

2002).

There are discrepancies among studies looking at lamb growth performance in

response to inclusion of Se in the diet. Reasons for the Se discrepancies include the

chemical form of Se administered, the supplementation rate, and the Se status of

treatment groups, the age of lambs studied, and the route of Se administration. The

NRC (2007) recommendations state that the highest Se requirement for lambs occurs

during periods of rapid physiological development, specifically the periods from birth

to weaning. Abd El-Ghany et al. (2008) and Gabryszuk and Klewiec (2002) suggest

that growth as a result of Se supplementation is most noticeable in the first two weeks

of age, although other studies suggest that enhanced growth from Se supplementation

can be detected in lambs up to one year of age (Munoz et al., 2008; Kumar et al.,

2009). In contrast, Dominguez-Vara et al. (2009) found no differences in growth

response in weaned lambs supplemented with 0.3 mg/kg SeY in the finishing diet,

when compared with non Se-supplemented lambs. Similar results were found with

McClure and Mahan (1988) and Christaldi et al. (2005) whereby no effect of Se

supplementation on growth rate was observed in growing and finishing lambs

supplemented with increasing amounts of sodium selenite. The lack of effect of Se on

growth in studies conducted by Christaldi et al. (2005), Dominguez-Vara et al.

(2009), and McClure and Mahan (1988) might be the result of using older age lambs compared to the younger age of lambs in studies that did observe improved growth responses from Se supplementation. It is possible that young lambs are more sensitive to Se supplementation than lambs reaching physiological maturity.

2.2.3 Maternal Transfer of Se to Fetus

It is important to ensure that ewes receive adequate Se supplementation during periods of rapid fetal growth, i.e., during late gestation and in early lactation. Abd El-Ghany et al. (2007) found that maternal plasma and liver Se concentrations decreased from early pregnancy to late pregnancy. At the same time, fetal liver and kidney Se concentrations increased from early to late pregnancy. Placental and colostral transfer of Se from ewe to lamb has been shown to occur even when the dam is deficient in Se (Abd El-Ghany et al., 2007, 2008, 2010). The decrease in maternal plasma-Se concentrations and the relative increase in fetal-Se concentrations suggest that demand for Se increases as gestation progresses. It is possible to ensure that lambs have adequate Se at birth and throughout the growing period by providing supranutritional Se to the ewe prior to parturition (e.g., perinatal supplementation).

The efficiency of placental transfer is highly dependant on the chemical form of Se supplemented. Organic Se sources are more readily transferred than inorganic sources (Kincaid and Rock, 1999). According to Taylor et al. (2009) ewes fed Se-enriched wheat grain had greater placental transfer of Se to the fetus compared with ewes supplemented with sodium selenate. Ewes required approximately 5 times as much sodium selenate to equal the amount of Se transferred to fetus of ewes fed Se-

enriched wheat grain. Taylor et al. (2009) also observed that the placenta limited the amount of Se transferred to the fetus when Se was fed as sodium selenate, whereas Se from Se-enriched wheat was observed to accumulate in the fetus in greater concentrations.

Selenium in colostrum and milk are also important Se sources for the newborn lamb. Colostral Se-concentrations have been shown to increase linearly with increasing dietary Se concentrations (Abd El-Ghany et al., 2007, 2008; Davis et al., 2006). Davis et al. (2006) observed greater Se concentrations in colostrum (705 ng/mL decreasing to 57 ng/mL at day 3 of lactation). The higher Se concentrations in colostrum were reflected in lamb plasma-Se concentrations peaking at 3 days of age. Peak lactation for a mature ewe nursing twin lambs occurs at approximately 21 days after parturition (Cardellino and Benson, 2006). Interestingly, Se concentrations in the milk decline after approximately 3 weeks of lactation (Davis et al., 2006). The rate of decline in milk-Se concentrations was more rapid in ewes supplemented with sodium selenite at less than 4 mg/kg compared with ewes supplemented at 8 to 20 mg/kg (Davis et al., 2006).

2.3   THE EFFECT OF SELENIUM ON IMMUNE FUNCTION

2.3.1 Overview of the Immune System

The immune system protects animals against microbial invasion and is necessary for survival. The immune system can be divided into two parts: innate immunity and acquired immunity. Innate immunity is always "on" and provides rapid initial protection to an animal. Innate immunity focuses it defense mechanisms at

sites of microbial invasion. Cells involved in innate immunity are neutrophils, macrophages, dendritic cells, and natural killer cells, which kill invading micro-organisms. Even though the innate immune system is always on, it responds identically to invading organisms and can be overwhelmed. Also, it does not form memory to invading antigens and, therefore, its effectiveness does not improve over time.

The innate immune system is necessary for animal survival but is only the first line of defense. The acquired immune system provides an ever improving long-term solution to identifying and destroying invaders. In contrast to the innate immune system, the acquired immune system is activated by antigens and takes several days to become fully effective. The acquired immune system not only recognizes and destroys a wider array of invading microorganisms than the innate system, but also can remember the encounter.

The acquired immune system can be further classified into two immune responses. The first is the humoral immune response, which is directed against extracellular organisms (e.g., bacteria, fungi, and protozoa), and uses antibodies to mediate killing. The second is the cell-mediated immune response, which is directed against intracellular organisms (e.g., viruses and protozoa) and uses specialized T and B cells (Tizard, 2009).

2.3.2 How Se Effects the Immune System

Selenium's pivotal role in animal health centers on its role as an antioxidant. As an essential component of the glutathione peroxidase (GHPx) family of enzymes,

Se exerts its influence by preventing the degradation of DNA, proteins, lipids, and carbohydrates caused by reactive oxygen species (ROS). Although ROS and free radicals are a natural result of the body's normal metabolic activity, excessive stress as a result of disease, environmental extremes, and nutritional imbalances can lead to over production of free radicals. Therefore, it is imperative that antioxidant components (e.g., Se, Mn, Cu, Zn, and Vitamins E, A, and C) are present in tissues to provide oxidant-antioxidant balance (Surai, 2006). Selenium's role in immune function is mainly a result of its function as an anti-oxidant.

Although Se deficiency does not affect the number of nuetrophils, it does impair aspects of their function. Neutrophils and macrophages kill bacteria by generating superoxide and hydrogen peroxide in the respiratory burst process (Rooke et al., 2004; Tizard, 2009). Although effective at killing bacteria, these peroxides also have the ability to cause cellular damage if not quenched after killing. Selenium deficiency impairs the ability of certain GHPx enzymes ability to metabolize peroxides and prevent self-inflicted damage (Arthur, 2003).

Cell-mediated immune responses develop as a result of interactions between antigens and immune cells. Signaling molecules such as cytokines bind to target receptors on other immune cells (Tizard, 2009). Selenium enhances the ability of lymphocytes to respond to the cytokine IL-2 by increasing the expression of IL-2 receptors on lymphocytes (Rooke et al., 2004). Enhancement of these interactions leads to increased numbers of lymphocytes, increased cytotoxicity of killer cells, and increased antibody production by B cells (Rooke et al., 2004; Tizard, 2009). Turner

and Finch (1991) observed impaired bursal growth in Se-deficient chicks and an overall reduction in the number of lymphocytes in the thymus and bursa. Because T and B cell maturation and training is dependant on proper functioning of these primary lymphoid organs, Se supplementation is particularly important in immature animals with developing immune systems. A comprehensive review by Rooke et al. (2004) that covered Se supplementation's effect on antibody production in mature ewes found increases in specific antibody production to: *tetanus toxoid, parainfluenza type-3, Corynebacterium psuedotuberculosis, Chlamydia psittaci, Brucella ovis, and Clostridium perfringins*. Enhanced cell-mediated immunity against the poliovirus in humans supplemented with Se was observed by Broome et al. (2004). Selenium supplementation in humans with low Se status resulted in increased production of IFN-γ and IL-10, an earlier peak in T-cell proliferation, and a more rapid clearance of the virus (Broome et al., 2004).

The relationship between Se status and immunity appears to be more complex than the generalized notion that Se supplementation boosts the immune response. A review by Hoffman and Berry (2008) suggests that immune enhancement depends on the types of antigens and tissues involved. Discrepancies amongst findings in human studies looking at the role of Se in enhancing immune function raise questions as to whether Se status is directly involved or instead represents part of an overall enhanced nutritional status (Drain et al., 2006). Hoffman and Berry (2008) suggest that certain infectious agents may actually benefit from added Se by utilizing it for their own antioxidant enzymes, although this theory is not well defined. Another

important consideration when looking at Se and its effects on immune function is the pre-existing Se status of the animal. Numerous studies suggest that the most noticeable immune enhancing effects are observed in animals that are Se deficient or moderately deficient compared to animals with already adequate Se status (Rooke et al., 2004).

2.3.3 The Importance of Selenium for Passive Immunity

Transplacental transfer of maternal antibodies, or immunoglobulins, is completely inhibited in ruminant species that have a syndesmochorial placenta (Tizard, 2009). Thus, passive immunity, or the transfer of maternal antibodies from dam to offspring via the placenta or ingested colostrum, occurs only by the latter route. Passively transferred immunoglobulins are essential for newborn survival, especially during the first weeks of life, and provide the primary means of defense against harmful pathogens.

Maternal immunoglobulins are a reflection of the immunological experiences of the mother. During the last 12 days of gestation the serum immunoglobulin concentrations in the ewe begin to decrease as large amounts of immunoglobulins are transferred to the mammary gland (Campbell et al., 1977; Rodinova et al., 2008). Colostrum is the first secretion of the mammary gland following parturition and is important not only for energy needed to keep lambs warm, but also for its antibody content. Colostrum contains approximately 7% fat, 4% casein, 5% lactose, and 82% water. It provides approximately 2 Kcal of energy per mL. It is estimated that 180 to 290 mL/kg BW are required by the lamb in the first 18 hours after birth (Nowak and

Poindron, 2006). Colostrum is saturated with immunglobulins (IgG, IgA, IgM), although the predominant type is IgG (approximately 60 to 90%) (Tizard, 2009).

A low level of protease activity in the digestive tract of newborn ruminants allows colostral proteins to reach the small intestine intact. In the small intestine, colostral immunoglobulins bind to specialized receptors on epithelial cells. They are subsequently endocytosed by intestinal epithelial cells and eventually reach the blood stream (Tizard, 2009). Increased intestinal permeability for colostral IgG is restricted to the first 24 to 48 hour period following parturition. Thus, it is important that lambs consume adequate amounts of colostrum in a timely manner to receive the transfusion of maternal antibodies.

The absorption of colostral immunoglobulins in the small intestine and subsequent transfer to the neonate's blood can be measured in lamb serum. Lamb IgG concentrations are most commonly measured at 12, 36, and 48 hours after parturition, and values range from 15 mg/mL to 87 mg/mL (Christley et al., 2003; Gilbert et al., 1988; French et al., 1996). Immunoglobulin G values less than 15 mg/mL constitute failure of passive transfer (Hunter et al., 1977). A lack of IgG in neonatal lamb serum has been associated with increased disease susceptibility and death losses (Campbell et al., 1977; Christley et al., 2003; Gilbert et al., 1988). McGuire et al. (1983) found that failure of passive transfer in neonatal lambs resulted in 45% dying before 3 weeks of age, whereas only 5% of the lambs with adequate passive transfer died. Failure of passive transfer can generally be attributed to poor colostrum quality, inadequate colostrum ingestion, or intestinal absorption failure. Specific factors such

as prematurity, dystocia, and congenital defects also affect the quantity of colostrum ingested (Christley et al., 2003; Gilbert et al., 1988).

Studies have been conducted to quantify the acceptable range for colostral IgG concentrations in ewes. A study conducted at Oregon State University using ewes of similar genetic makeup showed that colostral IgG concentrations measured by radial-immuno diffusion (RID) were 79 ± 5.6 mg/mL (Al-Sabbagh et al., 1995). Another study showed IgG concentrations to be 80 ± 2.4 mg/mL in ewe colostrum (Gilbert et al., 1988). Hunter et al. (1977) showed that Se concentrations in ewe colostrum were as high as 115.1 ± 10.1 mg/mL. It is important to note that IgG concentration in ewe's colostrum decreases rapidly after parturition.

Immunoglobulin concentrations in ewe's milk are affected by multiple factors such as sire breed, ewe age, and number of developing fetuses. Gilbert et al. (1988) showed that the more prolific breeds such as Polypays have higher colostral IgG concentrations, suggesting that as the number of fetuses increases so does the amount of IgG partitioned to colostrum. Gilbert et al. (1988) also showed that age affects IgG concentrations in colostrum with yearling ewes having the highest colostral concentrations at 100 mg/mL, 2 to 6 year old ewes having lower concentrations (65 to 67 mg/mL), and old- ewes greater than 7 years of age having the lowest IgG concentrations in colostrum (53 mg/mL).

Adequate Se status of the newborn not only ensures prevention of nutritional myopathy, but also decreases associated losses in lamb productivity. Lambs from Se-supplemented ewes show faster progression to stand and nurse (i.e., increased lamb

vigor) compared to lambs from non Se-supplemented ewes leading to an overall decrease in lamb mortality (Munoz et al., 2008). Dwyer et al. (2001) also showed that the time it takes a newborn lamb to stand and suckle directly correlates to improved survival rates. Increased lamb vigor ensures that adequate colostrum is consumed for IgG absorption during the critical neonatal period.

Selenium's effect on enhanced IgG absorption is not only a function of increased lamb vigor and colostral quality. There may also be an effect of Se on the intestinal epithelium. The simple addition of sodium selenite to colostrum increased IgG absorption in dairy calves at 48 hours when compared to unsupplemented controls (Kamada et al., 2007). Interestingly, the optimal absorption was achieved when calves consumed 3.0 mg/kg of added Se in the colostrum, in the range 0.2 to 5.0 mg/kg of added Se (Kamada et al., 2007).

Few researchers have looked specifically at the effect of Se source and concentration on IgG production in the ewe and consequent absorption in the neonatal lamb. Rock et al. (2001) found that IgG colostral concentrations were unaffected by Se supplementation, yet there was increased IgG absorption in lambs from ewes supplemented with Se at 0.3 mg/kg of the diet compared to unsupplemented controls. No significant differences were observed when comparing sodium selenite to SeY.

Rodinová et al. (2008) conducted an 8-month study whereby ewes were fed 180 µg of sodium selenite vs. SeY per ewe per day. Blood samples were collected from ewes and newborn lambs on days 1, 3, 10, 30 and 60. Ewe colostral IgG concentrations were unaffected by Se source, although lambs from Se-supplemented

ewes had higher IgG serum concentrations on days 1, 3, 30 and 60 post-parturition.

Lambs from ewes receiving SeY supplements had higher IgG concentrations on days

30 and 60. Evidence from these two studies shows the importance of Se in increasing

serum IgG concentrations in lambs, although there is limited evidence that Se affects

colostral quality.

The two methods for IgG quantification that are most commonly used in

sheep are RID and enzyme-linked immuno absorbent assay (ELISA). Most studies

conducted prior to 1995 were conducted using RID, whereas after 1995, because of

its higher sensitivity, the use of ELISA technology became an attractive alternative.

In contrast to RID, the ELISA method is less expensive and faster, taking 3 to 4 hours

vs. 24 hours (Ansfield et al., 2000; Chigerwe et al., 2005). Both techniques have an

agreement rate of 94% (Lee et al., 2008).

CHAPTER 3. EFFECT OF SELENIUM SOURCE AND SUPPLEMENTATION RATE IN EWES ON SELENIUM TRANSFER FROM EWE TO LAMB

3.1   ABSTRACT

Selenium (Se) is an essential micronutrient of sheep. Supplementation is especially important in young lambs in order to prevent Se-deficiency signs. In the United States, the FDA regulates Se supplementation to ruminant diets at a level of 0.3 mg/kg Se (as fed basis). To evaluate the effect of Se source and supplementation rate in ewes on Se status of their offspring, 240 ewes were divided into 8 treatment groups and drenched weekly (at an amount equal to their summed daily intake) for one year, including during gestation and early lactation, with no Se (deficient); at recommended levels (0.3 mg/kg) with inorganic sodium selenite, sodium selenate, or organic Se-yeast; or at supranutritional levels (0.9 and 1.5 mg/kg) with sodium selenite or Se yeast. Selenium administered by weekly drenching of ewes during gestation and early lactation was effective at increasing Se concentrations in ewe colostrum and milk at 30 days in milk (DIM) and in improving the Se status of lambs (whole-blood and serum Se at birth, and skeletal-muscle Se at 14 days of age) ($P < 0.001$). Selenium concentrations in lacteal secretions and lambs were higher in ewes drenched with Se yeast compared to ewes drenched with inorganic Se sources ($P < 0.01$). Selenium concentrations in lacteal secretions and in lambs increased linearly with supranutritional concentrations of Se yeast ($P < 0.001$), whereas Se concentrations did not differ in ewes drenched with 0.9 or 1.5 mg/kg of inorganic sodium selenite ($P > 0.05$). We conclude that weekly oral drenching of ewes with Se

yeast during gestation and early lactation is an effective method for improving Se status of their offspring.

3.2 INTRODUCTION

Selenium was discovered as an essential micronutrient in 1958. After its discovery, researchers focused initially on the quantity of dietary Se needed to prevent diseases associated with Se deficiency. Selenium-responsive diseases, such as nutritional myopathy and Se-responsive unthriftiness (SeRU), can be prevented in young ruminants when adequate Se is provided (Muth et al., 1958). The current FDA recommendation is 0.3 mg Se/kg DM (FDA, 1987). The range of adequate Se supplementation is narrow, as toxicity signs occur when lambs are injected with sodium selenite above 1 mg/kg or are fed SeMet above 4 mg/kg of body weight (BW) (Tiwary et al., 2006). Current FDA regulations limit the amount of dietary Se added to 0.7 mg per head per day or to 90 mg Se/kg of free-choice mineral supplement (FDA, 1987). Higher Se dosages are considered supranutritional. However, current recommendations do not account for the chemical form of Se [e.g., sodium selenite, sodium selenate, or selenium yeast (SeY)] and its effect on bioavailability, which may change with dosage. The bioavailability of a trace mineral refers to the degree to which an ingested nutrient is absorbed and available to the body. Besides the chemical form of Se, other factors that influence Se bioavailability in sheep are diet composition, type of digestive system (e.g., ruminant, pre-ruminant), route of administration (diet, drench, injection), and production stage (Surai, 2006).

Selenium sources can be classified into two categories: inorganic and organic. The most common inorganic Se sources are sodium selenite and sodium selenate, which are usually provided in mineral premixes or are injected. Organic Se sources are seleno-amino acids [e.g., selenomethionine (SeMet) and selenocysteine (SeCys)], which are found in SeY or in feeds grown on Se-rich soils. Organic Se sources are more bioavailable than inorganic Se sources because sodium selenite and sodium selenate must first be converted to hydrogen selenide (H_2Se) and then to selenophosphate ($HSePO_3^{-2}$) before they can be utilized in selenoprotein synthesis. For example, $HSePO_3^{-2}$ reacts with tRNA-bound serinyl residues to give SeCys-bound tRNA from which SeCys is inserted co-translationally at loci encoded by specific UGA codons, to give selenoproteins (Rayman et al., 2008). In contrast, when SeMet is consumed it can be tran-selenated to SeCys (by analogy with the trans-sulfuration pathway). Seleno-cysteine is then converted to H_2Se by SeCys ß-lyase. Alternatively, SeMet can be incorporated into protein in place of methionine; thereby providing a Se depot (Rayman et al., 2008).

Provision of Se to the dam during gestation and early lactation is thought to be an effective method to meet Se requirements in newborn ruminants. Selenium efficiently crosses the placental barrier into fetal tissues, as well as enters mammary secretions, i.e., colostrum or milk (Abd El-Ghany et al., 2007; Rock et al., 2001), with a greater transfer efficiency from dietary organic Se versus inorganic sodium selenite (Rock et al., 2001; Taylor et al., 2006). The objective of this study was to evaluate the effect of Se source and supplementation rate in ewes on Se status of their offspring.

Our hypothesis was that weekly oral Se drenching of ewes would improve the Se status of their lambs, with a dose-response effect based on ewe supplementation rates (0.9 and 1.5 mg/kg compared to the recommended 0.3 mg/kg), and with a greater response for an organic SeY source compared to inorganic Na_2SeO_3 and Na_2SeO_4 sources.

3.3   MATERIALS AND METHODS

3.3.1   Animals and Study Design

Experimental procedures used in this study were approved by the Institutional Animal Care and Use Committees of Oregon State University. This was a prospective, placebo-controlled clinical trial of 12-months duration involving 240 mature ewes from three genotypes (Polypay, Suffolk, and crossbred). Ewes ranged in age and BW from 2 to 6 yr, and 51 to 93 kg, respectively. The experiments were conducted at the Oregon State University Sheep Center, Corvallis, Oregon.

Ewes were randomly assigned to 8 treatment groups (n = 30 each) based on Se supplementation rate (0, 0.3, 0.9 and 1.5 mg/kg) and source [sodium selenite, sodium selenate (0.3 mg/kg only), and SeY]. Treatment groups were blocked for foot rot (FR) incidence and severity; breed; and age of ewe. The four dose levels (0, 0.3, 0.9, and 1.5 mg/kg) corresponded to no Se supplementation (0 mg/kg); 0.7 mg/d or 1x the FDA allowed supplementation rate (0.3 mg/kg); 2.1 mg/d or 3x the FDA allowed supplementation rate (0.9 mg/kg); and 3.5 mg/d or 5x the FDA allowed rate (1.5 mg/kg). All dosages were below the maximum tolerable level (5 mg/kg) for small ruminants (NRC, 2007).

3.3.2 Selenium Sources

Two inorganic Se sources were used: sodium selenite and sodium selenate, both from the same source (RETORTE Ulrich Scharrer GmbH, Röthenbach, Federal Republic of Germany). Sodium selenite was 456,000 mg/kg Se or 45.6% Se, and sodium selenate was 418,000 mg/kg Se or 41.8% Se (NRC, 2001). The organic Se source (SeY, Prince Se Yeast 2000, Prince Agri Products Inc., Quincy, IL) had a guaranteed analysis of 2,000 mg/kg of organically bound Se. Selenium supplements were solublized in water and drenched weekly. The calculated amount of Se delivered in the 0.3, 0.9 and 1.5 mg/kg weekly drench was 4.9, 14.7, and 24.5 mg Se per dose. Each composited drench was submitted for Se analysis (Center for Nutrition, Diagnostic Center for Population and Animal Health, Michigan State University, E. Lansing, MI) to verify the desired solution concentration. The 0.3 mg/kg weekly drench of inorganic sodium selenate was 82.5% higher (8.95 mg per dose) than targeted concentrations. The 0.3, 0.9 and 1.5 mg/kg weekly drenches of sodium selenite (4.85, 14.85, and 24.6 mg per dose, respectively) and organic SeY (1959 µg/g) were found to be within expected analytical variance of their targeted concentrations.

3.3.3 Selenium Administration

Selenium treatments were administered individually by oral drenching once weekly at an amount equal to the summed daily intake. Non Se-supplemented ewes received water. The Se dose was suspended in a reasonable volume of water (5 mL

for inorganic Se; more water was needed for the organic solutions, i.e., 11, 30 and 48 mL for 0.3, 0.9, and 1.5 mg/kg solutions, respectively), made up fresh each week, and administered with a dose syringe as sheep moved through a cutting chute. Color coding of Se sources to match ewe ear tags was utilized to maintain dosing accuracy. Sheep were treated once weekly for a total of 52 weeks. Lambs did not receive any additional Se supplementation after birth.

3.3.4   Sample Collection from Ewes and Lambs

Jugular venous blood was collected directly from all ewes every 3 months, and in 16 of 30 ewes of the no Se and 0.3 mg/kg dose groups every month, starting at study initiation. Immediately after parturition and before lambs had nursed, jugular venous blood was collected from lambs. For whole blood analysis, blood was collected into evacuated EDTA tubes (2 mL; final EDTA concentration 2 g/L; Becton Dickinson, Franklin Lakes, NJ) and stored on ice until it could be frozen at -20 ℃. For serum analysis, blood was collected into evacuated tubes without EDTA (10 mL; Becton Dickinson). Tubes were centrifuged at 850 x g for 10 min; serum was collected, transferred into 2.0 mL screw cap self-standing micro tubes (ISC BioExpress, Kaysville, UT) and stored at -20 ℃.

Colostrum samples were collected immediately following parturition. Milk samples were collected again 30 days after parturition. Ewes were milked by hand and samples were collected into 15 mL centrifuge tubes (10 mL; ISC BioExpress, Kaysville, UT) and stored at -20 ℃. Skeletal-muscle samples were collected from lambs at 14 days of age coinciding with tail docking. Tails were docked using a hot-

docking method and skeletal muscle samples were collected from the coccygeal vertebrae. Once docked, muscle on the tail was scraped off with a scalpel, separating muscle tissue from vertebrae and fat tissue. Muscle tissue from each lamb was then placed in a screw-top microtube (1.5 mL, ISC BioExpress, Kaysville, UT) and stored at -20 °C.

3.3.5   Se Analysis of Ewe and Lamb Samples

Selenium concentrations in whole blood, colostrum, milk, and muscle samples were determined by a commercial laboratory (Center for Nutrition, Diagnostic Center for Population and Animal Health, Michigan State University, E. Lansing, MI) using an ICP-MS method (Whalen et al., 2005) with modifications. Two-hundred μL of each whole-blood or serum sample was diluted 1:20 with a solution containing 0.5% EDTA and Triton X-100, 1% ammonia hydroxide, 2% propanol and 20 ppb of scandium, rhodium, indium and bismuth as internal standards. All samples were analyzed on an Agilent 7500ce ionized coupled plasma mass spectrometer. Selenium, at mass 78, was analyzed in hydrogen mode to reduce spectral interference.

3.3.6   Statistical Analysis

Statistical analyses were performed using SAS, version 9.1 (SAS, Inc., Cary, NC, USA) software. Ewes that did not give birth or rear a lamb were excluded from the statistical analysis (no Se = 3 ewes; 0.3 mg/kg sodium selenate = 2 ewes; 0.3 mg/kg sodium selenite = 4 ewes; 0.9 mg/kg sodium selenite = 4 ewes; 1.5 mg/kg sodium selenite = 2 ewes; 0.3 mg/kg SeY = 3 ewes, 0.9 mg/kg SeY = 4 ewes, 1.5 mg/kg SeY = 7 ewes). Values for multiple lambs from the same ewe were averaged

because ewe was the experimental unit. The effect of source and amount of Se supplement (no Se, 0.3 mg/kg sodium selenate, 0.3 mg/kg sodium selenite, 0.9 mg/kg sodium selenite, 1.5 mg/kg sodium selenite, 0.3 mg/kg SeY, 0.9 mg/kg SeY, 1.5 mg/kg SeY) on Se concentrations of whole blood, serum, colostrum, and 30-day milk in ewes; and of whole blood and serum at birth, and muscle at 14-days of age in lambs were analyzed using PROC GLM. Covariates in the model were FR-status (yes, no), breed (Polypay, Suffolk or crossbred), number of lambs born (1, >1). To evaluate whether FR-status, breed, number of lambs born, and number of lambs reared (1, >1) modified the effect of Se source and amount on blood-Se concentrations, data were additionally stratified by FR-status, breed, number of lambs born, and number of lambs reared, respectively.

The effect of no-Se supplementation on blood Se was evaluated by comparing the estimated values of the no-Se group with those dosed at the 0.3 mg/kg level. The effect of Se source was evaluated by comparing the estimated values of different Se sources at the same Se dosage. The effect of Se supplementation rate was evaluated by comparing the estimated values of different Se dosages within the same Se source. Data are reported as least square means ± SEM. Statistical significance was declared at $P \leq 0.05$.

3.4   RESULTS

3.4.1   Effect of No Selenium Supplementation

Compared to ewes that received 0.3 mg/kg Se, ewes receiving no Se supplementation had lower whole-blood Se in the first month of lactation (69% lower

compared to sodium selenate; 64% lower compared to Sodium selenite; 73% lower compared to SeY; **Figure 1A**) and lower serum-Se concentrations (61% lower compared to sodium selenate; 55% lower compared to sodium selenite; 63% lower compared to SeY; **Figure 1B**) (all $P < 0.0001$; **Table 1**). The decrease in whole-blood Se was greater than in serum Se concentrations (**Table 1**). In comparison to blood, changes in colostral Se in response to Se supplementation were larger, and changes in milk Se were smaller (**Table 1**). Compared to ewes being drenched with 0.3 mg/kg Se during gestation, ewes receiving no Se supplementation had much lower colostral Se concentrations (86% lower compared to sodium selenate; 81% lower compared to sodium selenite; 90% lower compared to SeY; all $P < 0.0001$; **Figure 1C**) and smaller changes in milk Se (47% lower compared to sodium selenate, $P = 0.02$; 35% lower compared to sodium selenite, $P = 0.17$; 61% lower compared to SeY, $P < 0.0001$; **Figure 1D**). Only non-Se-supplemented ewes had colostral-Se concentrations that were lower than serum-Se concentrations (**Figures 1B, 1C**). Otherwise, ewes receiving Se supplementation had higher colostral Se concentrations compared with their serum-Se concentrations.

Table 1. Whole-blood, colostrum-, and milk-Se concentrations (least squared means ± SEM) for ewes dosed weekly with inorganic or organic Se sources at varying dietary concentrations, and the corresponding whole-blood, serum, and skeletal-muscle Se concentrations of their lambs.

Se Source	Se dose (mg/kg)		Se Concentrations of Ewes					Se Concentrations of Lambs		
		N	Whole Blood[1] (Early Lactation) (ng/mL)	Serum[2] (Early Lactation) (ng/mL)	Colostrum (At Parturition) (ng/mL)	Milk (At 30 Days) (ng/mL)	N	Whole Blood (At Birth) (ng/mL)	Serum (At Birth) (ng/mL)	Muscle (At 14 Days) (ng/g)
No Se	0	27	103.2 ± 10.7^f	52.7 ± 3.7^g	38.5 ± 28.6^f	10.2 ± 2.8^e	27	99.49 ± 14.7^g	35.5 ± 4.3^g	191.9 ± 68.0^e
Selenate	0.3	28	327.7 ± 10.3^d	135.0 ± 3.5^e	280.8 ± 27.9^d	19.1 ± 2.8^{cd}	28	261.3 ± 14.2^e	66.3 ± 4.2^{ef}	369.5 ± 63.6^{de}
Selenite	0.3	26	289.3 ± 10.9^e	118.4 ± 3.7^f	203.8 ± 29.3^e	15.7 ± 2.9^{de}	26	221.2 ± 15.0^f	58.8 ± 4.5^f	304.6 ± 69.1^{de}
	0.9	26	366.5 ± 10.9^c	150.0 ± 3.7^d	334.4 ± 29.4^{cd}	20.9 ± 2.9^{cd}	26	304.4 ± 15.0^d	77.1 ± 4.4^{de}	442.0 ± 68.4^d
	1.5	27	386.1 ± 10.6^c	166.9 ± 3.6^c	427.6 ± 29.1^b	24.2 ± 2.8^c	27	332.3 ± 14.9^d	84.6 ± 4.4^d	482.1 ± 67.9^d
Se Yeast	0.3	27	378.6 ± 10.4^c	144.3 ± 3.6^{de}	373.6 ± 28.4^{bc}	26.1 ± 2.8^c	27	406.6 ± 14.2^c	98.3 ± 4.2^c	709.6 ± 64.8^c
	0.9	26	521.6 ± 10.6^b	182.0 ± 3.6^b	435.8 ± 30.3^b	38.7 ± 2.9^b	26	558.3 ± 14.6^b	125.9 ± 4.2^b	990.7 ± 67.8^b
	1.5	23	703.8 ± 11.3^a	217.0 ± 3.9^a	981.8 ± 31.1^a	63.7 ± 3.1^a	23	695.3 ± 15.6^a	182.6 ± 4.6^a	1695.7 ± 69.8^a

[a,b,c,d,e,f,g,] Within a column, means without a common superscript differ ($P < 0.05$).

[1] Ewe whole-blood samples were collected at approximately 2 weeks into lactation.

[2] Ewe serum samples were collected at approximately 2 weeks into lactation.

Figure 1. Effect of no Se supplementation and Se supplementation from two inorganic sources and one organic source on Se concentrations (least-squares mean ± SEM) of **A**) whole blood (< 30 DIM), **B**) serum (<30 DIM), **C**) colostrum, and **D**) milk (30 DIM) of the ewe; and **E**) whole blood (at birth), **F**) serum (at birth), and **G**) lamb skeletal muscle (at 14 days). [a,b,c,d] Bars without a common superscript differ ($P < 0.05$).
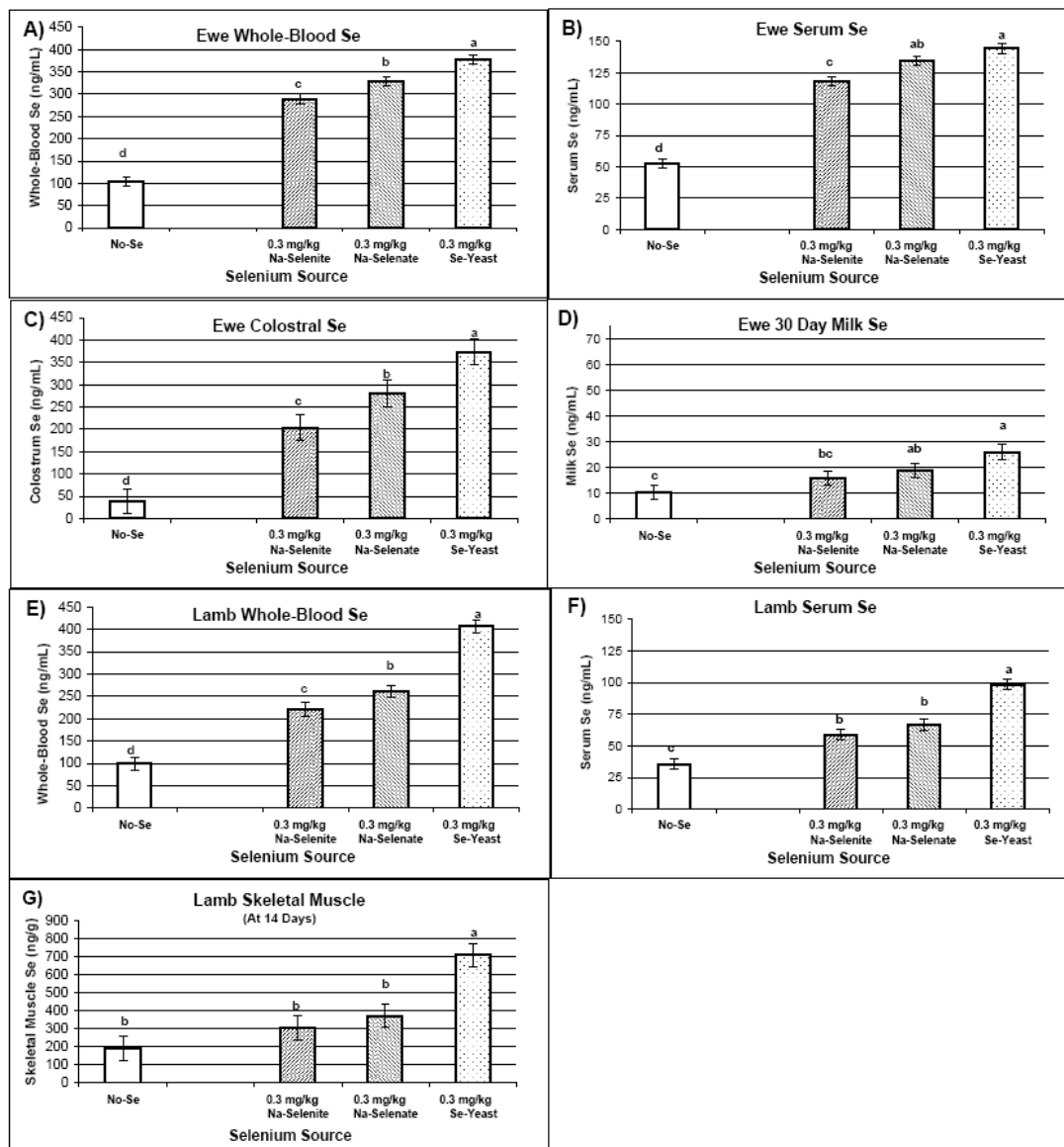
Figure 2 Effect of supranutritional Se supplementation with Na-selenite or Se-Yeast at increasing dosages on Se-transfer efficiency ratios (least-squares mean ± SEM) for **A**) ewe colostrum to ewe whole-blood (<30 DIM), **B**) ewe colostrum to ewe serum (<30 DIM), **C**) lamb whole-blood to ewe whole-blood, **D**) lamb serum to ewe serum, **E**) lamb skeletal-muscle (at 14 days) to ewe whole-blood, **F**) lamb skeletal-muscle (at 14 days) to ewe serum, and **G**) lamb skeletal-muscle (at 14 days) to ewe colostrum. [a,b,c,d] Bars without a common superscript differ ($P < 0.05$).
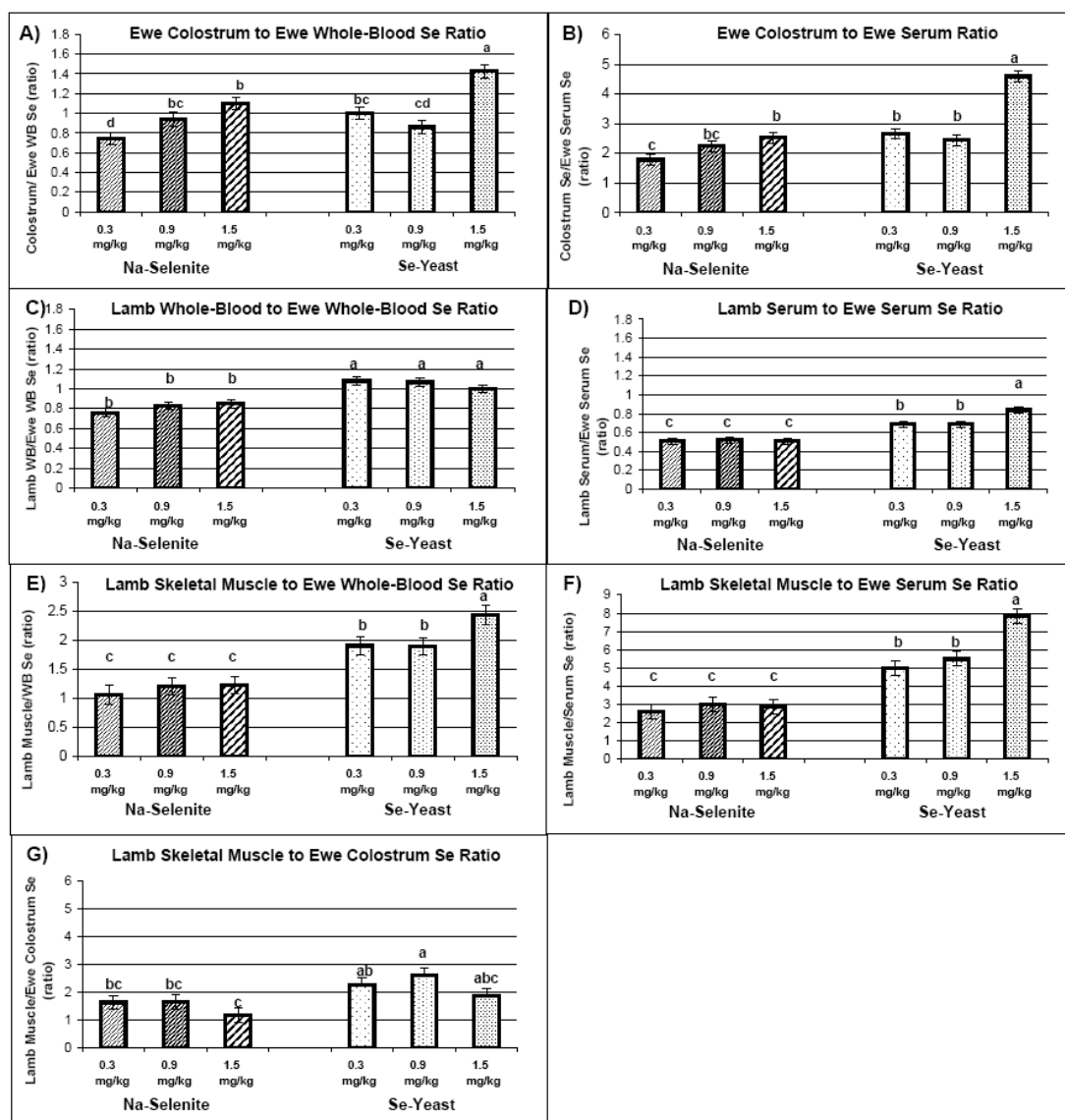
Changes in blood Se concentrations in response to Se supplementation were similar in ewes and their offspring (**Table 1**). Lambs from ewes receiving no Se supplementation had lower whole-blood Se concentrations at birth (62% lower compared to sodium selenate; 55% lower compared to sodium selenite; 76% lower compared to SeY) Se concentrations at birth (all $P < 0.0001$; **Figure 1E**). Furthermore, serum-Se concentrations were also lower (46% lower compared to sodium selenate; 40% lower compared to sodium selenite; 64% lower compared to SeY) in lambs (all $P < 0.0001$; **Figure 1F**); although not to the same degree as whole-blood Se concentrations of lambs. In ewes receiving no Se, ewes and their offspring had similar whole-blood Se concentrations (**Table 1**). In contrast, serum Se concentrations were higher in ewes than in their offspring; thus, indicating that lambs had more non-serum Se than ewes (**Table 1**). Changes in lamb-skeletal muscle at 14 days of age followed similar trends as other Se measures in ewes and lambs (**Table 1**). Lambs from ewes receiving no Se had significantly lower skeletal muscle Se concentrations (-73%) than lambs from ewes receiving 0.3 mg/kg SeY ($P < 0.0001$), yet only numerically lower Se concentrations compared to lambs from ewes receiving sodium selenate (-48%; $P = 0.05$) or sodium selenite (-37%; $P = 0.23$; **Figure 1G**).

3.4.2 Effect of Supplementing with Different Selenium Sources at the FDA-Recommended Concentration

At the FDA-recommended concentration of 0.3 mg Se/kg DM, organic SeY resulted in higher blood-Se concentrations in ewes than inorganic sodium selenite (31% greater for whole blood; 22% greater for serum (both $P < 0.0001$) and inorganic sodium selenate (16% greater for whole blood, $P = 0.0005$; 7% numerically greater for serum, P

= 0.06; **Figures 1A, 1B**). The SeY-induced Se increases were greater in colostrum and milk (**Table 1**). Specifically, colostral Se concentrations in SeY-supplemented ewes were 83% higher compared to sodium selenite ($P < 0.0001$) and 33% higher compared to sodium selenate ($P = 0.02$; **Figure 1C**); and milk Se concentrations were 67% higher compared to sodium selenite ($P = 0.01$); and 37% numerically higher compared to sodium selenate ($P = 0.08$; **Figure 1D**). Inorganic Se in the form of sodium selenate was more effective in increasing Se concentrations in blood (13% higher with $P = 0.009$ for whole blood; 14% higher with $P = 0.001$ for serum) and lacteal secretions (38% higher with $P = 0.05$ for colostrum; 22% numerically higher with $P = 0.40$ for milk) than sodium selenite; although the effect was not significant for milk because of large coefficients of variation (**Figure 1D**).

Similar changes in blood-Se concentrations were observed in ewes and their offspring (**Table 1**). In addition, organic SeY had a higher transfer efficiency from ewe to lamb blood than inorganic Se (**Figure 2C, 2D**). Lambs of SeY-supplemented ewes had greater whole-blood concentrations than their dams (7%), while lambs receiving inorganic sodium selenite (-24%) or sodium selenate (-20%) had lower whole-blood concentrations than their dams (**Table 1**). A similar directional trend was observed for lamb versus ewe serum concentrations (-32% for SeY group; -50% for sodium selenite group; and -51% for sodium selenate group; **Table 1**). The ewes' Se source did not alter in newborn lambs the fraction of Se that was found in serum (**Table 1**). Muscle-Se concentrations of 14- day-old lambs reflected ewe whole-blood Se concentrations in early lactation (**Figure 1**) and ewe colostral-Se concentrations (**Table 1**). In addition, ewes receiving SeY transferred a higher proportion of Se into muscle than ewes receiving

inorganic Se (**Figure 2E, 2F**). Lambs of SeY-supplemented ewes had greater skeletal-muscle Se concentrations compared to lambs of ewes receiving inorganic Se (133% higher compared to sodium selenite, $P < 0.0001$; 92% higher compared to sodium selenate, $P = 0.0002$; **Figure 1G**). In comparison, smaller differences were observed for lamb-whole blood (84% higher concentrations in lambs of ewes receiving SeY compared to sodium selenite, and 56% higher compared to sodium selenate; both $P < 0.0001$; **Figure 1E**), lamb serum (67% higher concentration in lamb serum of ewes receiving SeY compared to sodium selenite, and 48% higher compared to sodium selenate; both $P < 0.0001$; **Figure 1F**), and ewe colostrum (83% higher concentration in colostrum of ewes receiving SeY compared to sodium selenite, $P < 0.0001$; 33% higher compared to sodium selenate, $P = 0.02$; **Figure 1C**).

3.4.3   Effect of Supplementing with Sodium Selenite or Selenium Yeast at Increasing Supranutritional-Dosage Rates

Supranutritional supplementation rates for sodium selenite (combined 0.9 and 1.5 mg/kg ewe treatment groups) increased whole-blood (30% higher than for supplementation with the FDA recommended 0.3 mg/kg level; **Figure 3A**) and serum- (34% higher than for the 0.3 mg/kg level; **Figure 3B**) Se concentrations in ewes (both $P < 0.0001$). Even greater increases in whole-blood (62% higher than for the 0.3 mg/kg of SeY level) and serum- (38% higher than for the 0.3 mg/kg of SeY level) Se concentrations were achieved in ewes with supranutritional SeY supplementation (both $P < 0.0001$; **Figures 3A, 3B**). When supplementation rates were increased from 0.9 to 1.5 mg/kg of sodium selenite, increases in whole-blood (5%; $P = 0.18$) and serum-Se concentrations (11%; $P = 0.0009$) were smaller, and for whole blood non significant, than when supplementation rates were increased from 0.3 to 0.9 mg/kg (27% for both whole

blood and serum; both $P < 0.0001$; **Figures 3A, 3B**). In contrast, raising SeY supplementation rates from 0.9 to 1.5 mg/kg resulted in similar increases in whole-blood (35%; $P < 0.0001$) and serum- Se concentrations (19%; $P < 0.0001$) as did raising supplementation rates from 0.3 to 0.9 mg/kg (38% increase in whole-blood and 26% increase in serum-Se concentrations; both $P < 0.0001$; **Figures 3A**. Selenium supplementation at 0.3 mg/kg of SeY resulted in similar whole-blood Se concentrations in ewes as did supplementation of 1.5 mg/kg of sodium selenite (**Figures 3A, 3B**).

Colostral and milk-Se concentrations exhibited similar changes in response to supranutritional concentrations of dietary Se (**Table 1**). Colostral Se concentrations increased 87% with supranutritional supplementations of sodium selenite (combined 0.9 and 1.5 mg/kg ewe treatment groups) and 90% with supranutritional supplementation of SeY (combined 0.9 and 1.5 mg/kg ewe treatment groups) (**Figure 3C**). Supranutritional levels of dietary Se increased the transfer efficiency of Se from blood into colostrum. Furthermore, this effect was even more pronounced when ewes received SeY. As a result, the difference in Se concentrations in colostrum and milk between ewes receiving organic versus inorganic Se was greater at supranutritional compared to FDA-recommended dietary Se concentrations (**Figures 3C, 3D**). Specifically, the dosage-dependent increase from 0.3 to 1.5 mg/kg was stronger in colostrum (163%; $P < 0.0001$) and milk (144%; $P < 0.0001$) for SeY-supplemented ewes than for ewes receiving sodium selenite at the same supplementation rates (110% in colostrum, $P = 0.002$; and 53% in milk, $P = 0.03$; **Table 1**).

Ewes supplemented with sodium selenite had a limited dosage range over which it increased lamb-Se concentrations (**Table 1**). Whole-blood and serum-Se concentrations

in neonatal lambs did not significantly differ between ewes receiving 0.9 and 1.5 mg/kg of sodium selenite (**Figures 3E, 3F**). In contrast, the dosage range over which SeY can increase Se concentrations in neonatal lamb blood extends at least to 1.5 mg/kg, as whole-blood (25%) and serum- (45%) Se concentrations continued to increase from 0.9 to 1.5 mg/kg of dietary SeY (both $P < 0.0001$; **Figures 3E, 3F**). In addition, whole-blood Se concentrations in lambs of ewes receiving SeY were similar to their dam's-Se concentrations (+8%, +7%, and 0% for lambs from ewes receiving dietary Se at 0.3, 0.9, and 1.5 mg/kg, respectively), whereas they were lower in ewes supplemented with sodium selenite (-10%, -18%, -16% for lambs from ewes receiving dietary Se at 0.3, 0.9, and 1.5 mg/kg, respectively; **Table 1**). Similar directional trends were observed in serum-Se concentrations for lambs from ewes receiving dietary Se at 0.3 mg/kg (-31% SeY versus -49% sodium selenite); at 0.9 mg/kg (-31% versus -48%); and at 1.5 mg/kg (-16% versus -49%), indicating a greater placental transfer efficiency of SeY (**Table 1**). As a result, Se concentrations in lambs from ewes receiving 0.3 mg/kg of SeY were 22% higher in whole blood ($P = 0.0003$) and 16% higher in serum ($P = 0.02$) than Se concentrations in lambs from ewes receiving 1.5 mg/kg of sodium selenite (**Figures 3E, 3F**).

Figure 3. Effect of supranutritional Se supplementation with Na-selenate or Se-Yeast at increasing dosages on Se concentrations (least-squares mean ± SEM) of **A**) ewe whole blood (< 30 DIM), **B**) ewe serum (<30 DIM), **C**) colostrum, and **D**) milk (30 DIM); and **E**) lamb whole blood (at birth), **F**) lamb serum (at birth), and **G**) lamb skeletal muscle (at 14 days). [a,b,c,d] Bars without a common superscript differ (*P* < 0.05).
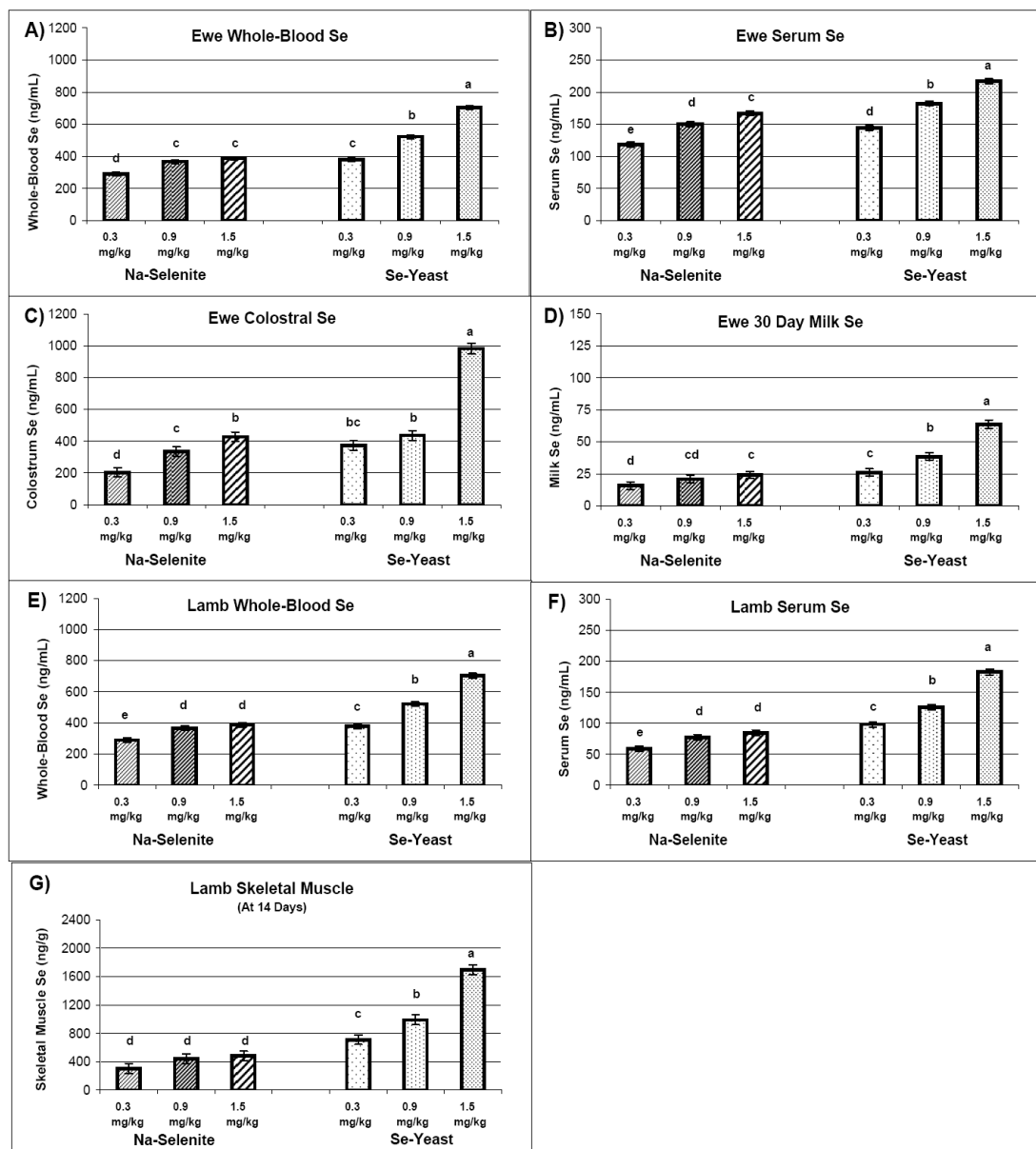
Lamb skeletal-muscle Se concentration at 14-days-of-age responded similarly to supranutritional Se supplementation with organic or inorganic Se sources as did other

indicators of Se status, with the gap in Se response further widening between sodium selenite and SeY supplementation (**Table 1**). The lamb-muscle Se response to sodium selenite supplementation started to plateau in lambs from ewes receiving 0.9 mg/kg of sodium selenite (442 versus 482 ng/g at 1.5 mg/kg; $P = 0.66$; **Figure 3G**). In contrast, the lamb-muscle Se response to SeY supplementation accelerated when ewe dosages were increased from 0.9 to 1.5 mg/kg (71% increase, $P < 0.0001$), compared to the increase from 0.3 to 0.9 mg/kg for SeY (40% increase, $P = 0.003$) (**Figure 3G**). As a result, muscle-Se concentrations in lambs from ewes receiving 0.3 mg/kg of SeY were 47% higher ($P = 0.01$) than in lambs from ewes receiving 1.5 mg/kg of sodium selenite (**Figure 3G**). In comparison, serum-Se concentrations of ewes receiving 0.3 mg/kg of SeY were 14% lower ($P < 0.0001$) than serum-Se concentrations in ewes receiving 1.5 mg/kg of sodium selenite (**Figure 3B**). This comparison exemplifies the superior transfer efficiency of SeY compared to sodium selenite.

3.5 DISCUSSION

3.5.1. Placental Transfer

The purpose of the study was to determine the effects of Se source and supplementation rate on Se transfer from ewe to lamb. It is known that Se status of newborn lambs closely correlates to Se status of their mothers (Abd El-Ghany et al., 2007). Thus, transplacental transfer of Se is the primary source of Se in the newborn lamb prior to colostral ingestion. This fact was reinforced in our study by comparing Se concentrations in whole-blood and serum collected from lambs at parturition to the Se concentrations of their mothers. Transplacental transfer of Se from ewe to lamb was affected by the source of Se (chemical form) as well as the concentration of Se

administered. This was shown best in whole-blood and serum-Se measurements of lambs born to ewes receiving increasing supranutritional Se dosage rates of SeY. Lamb whole-blood and serum-Se concentrations in our study are consistent with previous findings of Davis et al. (2006) and Juniper et al. (2008), although our study is unique in that different sources of Se and ewe supplementation rates were studied to assess their effects on Se status of newborn lambs. Additionally, our study compared increasing supranutritional Se dosages to ewes that were closer to the current 0.3 mg/kg NRC recommendations (i.e., 0.9 mg/kg and 1.5 mg/kg) compared to other studies that were aimed at determining maximum tolerable concentrations (Davis et al., 2006; Tiwary et al., 2006).

Our results showed differences in the transplacental Se transfer capacities of sodium selenite and SeY. When comparing these two Se sources at equivalent ewe dosing concentrations (0.3, 0.9 and 1.5 mg/kg), we observed that lambs from ewes receiving SeY had higher whole-blood and serum-Se concentrations compared to lambs from ewes receiving sodium selenite. Failure of sodium selenite to significantly increase newborn Se status when supplemented at increasing concentrations in our study agree with Behne et al. (2001) who suggests that inorganic Se sources are homeostatically controlled and blood levels cannot be significantly increased with increasing dosages. Our study findings are also consistent with Davis et al. (2006) who found that lambs from ewes fed dietary sodium selenite at 0.2, 4, 8, 12, 16 mg/kg did not have significantly different plasma Se concentrations. In contrast to sodium selenite, the placental transfer of Se from SeY significantly increased as the ewe supplementation rate increased from 0.3 mg/kg to 0.9 mg/kg, and from 0.9 mg/kg to 1.5 mg/kg, based on whole-blood and serum-Se concentrations of newborn lambs. Taylor et al. (2009) also observed

differences in the transferability between inorganic and organic Se sources, i.e., ewes fed Se from Se-enriched grain was more efficiently incorporated into fetal tissue than Se fed as sodium selenate. The greater transferability of SeY is the result of SeMet being the major Se-containing component in SeY (approximately 63% of total Se in SeY is SeMet) (Juniper et al., 2008). The biochemical similarity between SeMet and methionine (Met) allows non-specific interchange of SeMet for Met. In other words, the placenta cannot distinguish between SeMet and Met and, consequently, SeMet is transported to the fetus through an amino acid transporter rather than by Se-selective transport (Hawkes et al.,1994). For this reason, the capacity to accumulate Se in whole blood is greater in lambs from ewes supplemented with SeY compared to lambs from ewes supplemented with inorganic Se sources (e.g., sodium selenite and sodium selenate), which we showed with whole-blood data of ewes and lambs in our study.

Although not measured in our study, identification of the specific-Se species present in newborn lamb whole-blood and serum could help provide specific information about Se metabolism in pregnant ewes and neonatal lambs. Most of the Se from ingested sodium selenite is present as glutathione peroxidase (75 to 85%) in red blood cells and in the selenoprotein P fraction of serum (Tiwary et al., 2006). In contrast, when SeMet is consumed it is either non-specifically incorporated into hemoglobin in red blood cells as SeMet or it follows a specific metabolic pathway similar to selenite. Excess SeMet that is not immediately metabolized is incorporated non-specifically into proteins in place of Met as SeMet (Tiwary et al., 2006). Lambs from ewes fed 1.5 mg/kg SeY achieved the same 700 ng/mL whole-blood Se concentrations at birth that were attained in lambs from the Juniper et al. (2008) study that consumed 6.30 mg/kg of SeY for 91 days. This shows

that the developing fetus can accumulates Se relative to the amount of Se the dam consumes. Lamb whole-blood and serum-Se measurements in our study suggest that supranutritional Se supplementation to ewes throughout gestation is an effective method of elevating newborn Se status, and is best achieved with SeY containing Se sources.

3.5.2 Selenium in Colostrum and Milk

Results from our study emphasize the importance of colostrum as a source of concentrated Se. Regardless of the source of Se, our data shows that Se is transferred to colostrum and more so when ewes are supplemented with supranutritional concentrations of SeY. The same trends for SeY observed in transplacental transfer of Se were also observed for Se concentrations in colostrum. Ewes supplemented with SeY had 56% greater colostral Se concentrations than ewes supplemented with sodium selenite when compared at equal FDA-recommended concentrations. Selenium concentrations of colostrum increased as the concentration of the supplement increased irrespective of Se source consumed by the ewe, but overall the effect was more pronounced when ewes received SeY. Selenium concentrations in milk at 30 days were not much different amongst the inorganic selenite groups, yet were significantly increased in groups receiving SeY. Thus, SeY is more effectively transferred to milk at 30 days when compared to sodium selenite at equal supplementation rates. These finding are in agreement with Ortman and Pehrson (1999) who found that Se from SeY was more efficiently transferred to milk of dairy cows compared with sodium selenite when fed at equal concentrations. We can only speculate that higher Se concentrations in the milk of ewes in the 1.5 mg/kg SeY group are the result of SeMet being incorporated into milk proteins because of the relatively high Met content of milk (Weiss, 2005).

Our study provides new information with regards to how much Se is found in milk at 30 days lactation as a result of Se source and supplementation rate. Findings from our study suggest that Se concentrations of milk are low at 30 days lactation, compared to colostral-Se concentrations, especially in ewes supplemented at the recommended 0.3 mg/kg level. In the 1.5 mg/kg SeY group, the Se concentration in ewe milk at 30 days (63.7 ng/mL) contributed less to Se status of the lamb than colostral Se (981.8 ng/ml). Unique to our study was the finding that 30-day-milk-Se concentrations were significantly increased as a result of supranutritional (1.5 mg/kg) consumption of SeY. The limited ability to increase 30-day-milk-Se concentrations in ewes consuming sodium selenite further demonstrates the limited Se transfer capabilities of sodium selenite. This was also observed by Davis et al. (2006) in 28-day-milk samples from ewes consuming dietary sodium selenite at supranutritional concentrations (e.g., 0.2, 4, 8, 12, 16, 20 mg/kg). Based on data from Wohlt et al. (1984), twin lambs will consume approximately 900 g of milk per day at 30 days of age. Given this intake estimate, lambs consuming milk from ewes in our high SeY group (1.5 mg/kg) would ingest the equivalent of 0.06 mg of Se per day in milk at 30 days of age. Based on this calculation, and current NRC recommendations, it is improbable that sufficient Se can be supplied in milk to satisfy Se requirements at this stage of lamb growth. Furthermore, our findings and those of Davis et al. (2006) show that sodium selenite supplemented at 0.3, 0.9, and 1.5 mg/kg concentrations to ewes will not adequately provide required milk-Se concentrations at 30 days of age.

3.5.3   Selenium in Lamb Skeletal Muscle

Similar to whole-blood Se concentrations, Se concentrations in skeletal muscle were also increased in lambs from ewes supplemented with increasing Se concentrations, with the highest concentrations in lambs from ewes supplemented with SeY. Compared to lambs from ewes supplemented at the NRC recommended 0.3 mg/kg, lambs from ewes receiving SeY at 0.9 mg/kg, and 1.5 mg/kg had 29% and 59% higher Se concentrations in skeletal muscle, respectively. Ewes supplemented with Se from inorganic sources beyond the 0.9 mg/kg supplementation rate were not as effective at raising lamb skeletal-muscle Se concentrations. The lamb-muscle Se response to sodium selenite supplementation started to plateau in lambs from ewes receiving 0.9 mg/kg of sodium selenite (442 versus 482 ng/g at 1.5 mg/kg). Juniper et al. (2008) showed that lambs fed supranutritional levels of SeY (e.g., 6.3 mg/kg) for a period of 91 days achieved Se skeletal-muscle concentrations of 7.82 mg/kg. In contrast, our lambs from ewes receiving 1.5 mg/kg of SeY had skeletal muscle Se concentrations of 1695 ng/g or 1.7 mg/kg DM in comparison to Juniper et al. (2008). Based on fives times higher supplementation rates in the Juniper et al. (2009) study, and five times higher skeletal muscle Se concentrations in the Juniper et al. (2009) results compared to our results, it is likely that lambs from ewes supplemented with SeY accumulate SeMet in a dose-response relationship as the main Se type in skeletal muscle. An abundance of SeMet in skeletal muscle could serve as a Se storage pool to be drawn upon when needed for synthesis of specific seleno-proteins when supplemental Se is not available (NRC, 2007).

3.5.4 Implications for the Sheep Industry

This study has two major implications for the sheep industry. First, drenching of ewes with sodium selenite, although relatively cheap, is an ineffective method to improve Se status of newborn lambs. This is likely because of its low bioavailability and limited half-life (Whanger , 2002). Second, SeY drenching of pregnant ewes, although more expensive, provides an effective method to improve Se status of newborn lambs. The benefits for lambs born with adequate levels of Se are enhanced absorption of maternal antibodies in colostrum (Rock et al., 2000) and enhanced cell- mediated immune function, including better responses to vaccination (Rooke et al., 2004). Given that our oral 0.3 mg/kg SeY dosage is sufficient to provide Se for 210 days (Juniper et al., 2008), SeY drenching during the lambing period would provide sufficient Se to lambs, and eliminate the need for costly injections or mineral Se pre-mixes, which quickly lose efficacy when exposed to the environment. Oral SeY drenching would be especially useful to producers that raise animals in Se deficient regions for extended periods of time.

3.6 REFERENCES

See Chapter 7.

CHAPTER 4.   EFFECT OF SELENIUM SOURCE AND SUPPLEMENTATION RATE ON IMMUNOGLOBULIN G CONCENTRATIONS IN COLOSTRUM FROM EWES AND SERUM FROM LAMBS

4.1   ABSTRACT

Newborn lambs depend upon their dams for passive transfer of immunoglobulins, primarily immunoglobulin G (IgG), for protection from harmful pathogens until their own immunological defenses have developed. Previous studies, using small numbers of animals, have suggested that supplementation with Se results in a modest increase in IgG concentration in serum of newborn calves and lambs. To examine the effect of Se source and supplementation rate in ewes on colostral IgG and lamb serum-IgG concentrations, 240 ewes were divided into 8 treatment groups and drenched weekly (at an amount equal to their summed daily intake) for one year, including during gestation and early lactation, with no Se (deficient); at recommended levels (0.3 mg/kg) with inorganic sodium selenite, sodium selenate, or organic Se-yeast; or at supranutritional levels (0.9 and 1.5 mg/kg) with sodium selenite or Se yeast. Ewe colostrum and lamb serum-IgG concentrations were measured at parturition, and lamb serum-IgG concentration was measured again at 48 hours postnatal. Although Se drenching of ewes was effective at increasing whole-blood Se concentrations in ewes and lambs ($P < 0.001$), and colostral Se concentration ($P < 0.001$), there was no consistent or significant increase in IgG concentrations in ewe colostrum nor lamb serum at 48 hours of age ($P > 0.05$) irrespective of Se source, or supplementation rate. Therefore, we conclude that Se supplementation in ewes, and Se status of newborn lambs, have little effect on IgG

concentrations in colostrum and subsequent IgG concentrations in lamb serum at 48 hours postnatal.

4.2   INTRODUCTION

Newborn lamb survival is highly dependant upon the ability of the lamb to consume adequate amounts of colostrum in a timely manner. Transplacental transfer of maternal antibodies, or immunoglobulins, is completely inhibited in ruminant species that have a syndesmochorial placenta (Tizard, 2009). Thus, passive immunity, or the transfer of maternal antibodies from dam to offspring via the placenta or ingested colostrum occurs only by the latter route. Passively transferred immunoglobulins are essential for newborns especially during the first weeks of life and provide the primary means of defense against harmful pathogens.

Colostrum is the first secretion of the mammary gland following parturition and is important not only for the energy needed to keep lambs warm, but also for its immunoglobulin (Ig) content. Colostrum is saturated with immunglobulins (IgG, IgA, and IgM), although the predominant type is IgG (approximately 60 to 90%) (Tizard, 2009). Low levels of protease activity in the digestive tract of newborn ruminants allow colostral proteins to reach the small intestine intact. In the small intestine, colostral Ig binds to specialized receptors on epithelial cells. Receptor-bound Ig are subsequently endocytosed by intestinal epithelial cells and eventually reach the blood stream (Tizard, 2009). Increased intestinal permeability for colostral IgG is restricted to the first 24 to 48 hour period following parturition. A lack of IgG in neonatal lamb serum has been associated with increased disease susceptibility and death losses (Christley et al., 2003; Gilbert et al., 1988).

Selenium (Se) is well known as an essential trace mineral in sheep and cattle. Selenium was discovered as an essential micronutrient in 1958. After its discovery, researchers focused initially on the quantity of dietary Se needed to prevent Se-responsive diseases. More recently, researchers have shifted their focus to the source and supplementation rate of Se needed to maximize immune function. Questions still exist regarding what chemical form of Se [e.g., sodium selenite, sodium selenate, or selenium yeast (SeY)] is the most bioavailable supplement; whether the FDA recommended 0.3 mg/kg concentration is sufficient; and what is the best route to provide Se (e.g., mineral premix, injection, or oral drench). Current FDA regulations limit the amount of Se that can be added to complete feeds to 0.3 mg/kg, or no more than 0.7 mg per head per day. Dietary concentrations that exceed 0.3 mg/kg are referred to as supranutritional (NRC, 2007). Previous studies have documented that supranutritional dietary Se can be fed to sheep without causing signs of Se toxicity in pregnant ewes and newborn lambs (Davis et al., 2006; Reed et al., 2007; Taylor et al., 2005, 2009).

Selenium supplementation may boost passive immunity by enhancing IgG absorption in the newborn (Rock et al., 2001). Although passive immunity has been shown by others to be enhanced as a result of Se supplementation rates less than or equal to 0.3mg/kg, it remains unclear what effects different Se sources and supranutritional-Se supplementation rates have on passive immunity. Therefore, the objectives of this study were to determine the effects of Se source (e.g., sodium selenite, sodium selenate, or selenium yeast), and dietary-Se concentration (0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg) on ewe colostral IgG and lamb serum-IgG concentrations. It was hypothesized that ewes receiving supranutritional Se doses (i.e., 0.9 mg/kg and 1.5 mg/kg) of SeY or sodium

selenite would have greater colostral IgG concentrations than ewes not receiving Se, and greater colostral IgG concentrations than ewes supplemented at the recommended 0.3 mg/kg concentration. Furthermore, ewes supplemented with SeY at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg would have greater colostral IgG concentrations when compared with ewes receiving Se as sodium selenite at these respective concentrations. We also hypothesized that IgG concentration in serum would be greatest in lambs from ewes supplemented with supranutritional concentrations of Se (e.g., 0.9 mg/kg and 1.5 mg/kg) compared to lambs from ewes supplemented with Se at the 0.3 mg/kg concentrations or non-supplemented ewes. Furthermore, lambs from ewes supplemented with SeY would have greater IgG concentrations in serum than lambs from ewes supplemented with sodium selenite at their respective concentrations.

4.3   MATERIALS AND METHODS

4.3.1   Animals and Study Design

Experimental procedures used in this study were approved by the Institutional Animal Care and Use Committees of Oregon State University. This was a prospective, placebo-controlled clinical trial of 12-months duration involving 240 mature ewes from three genotypes (Polypay, Suffolk, and Crossbred). Ewes ranged in age and BW from 2 to 6 yr, and 51 to 93 kg, respectively. The experiments were conducted at the Oregon State University Sheep Center, Corvallis, Oregon.

Ewes were randomly assigned to 8 treatment groups (n = 30 each) based on Se supplementation rate (0, 0.3, 0.9 and 1.5 mg/kg) and source [sodium selenite, sodium selenate (0.3 mg/kg only), and SeY]. Treatment groups were blocked for foot rot (FR) incidence and severity; breed; and age of ewe. The four dose levels (0, 0.3, 0.9, and 1.5

mg/kg) correspond to no Se supplementation (0 mg/kg); 0.7 mg/d or 1x the FDA allowed supplementation rate (0.3 mg/kg); 2.1 mg/d or 3x the FDA allowed supplementation rate (0.9 mg/kg); and 3.5 mg/d or 5x the FDA allowed rate (1.5 mg/kg). All dosages were below the maximum tolerable level (5 mg/kg) for small ruminants (NRC, 2007).

4.3.2 Selenium Sources

Two inorganic Se sources were used: sodium selenite and sodium selenate, both from the same source (RETORTE Ulrich Scharrer GmbH, Röthenbach, Federal Republic of Germany). Sodium selenite was 456,000 mg/kg Se or 45.6% Se, and sodium selenate was 418,000 mg/kg Se or 41.8% Se (NRC, 2001). The organic Se source (SeY, Prince Se Yeast 2000, Prince Agri Products Inc., Quincy, IL) had a guaranteed analysis of 2,000 mg/kg of organically bound Se. Selenium supplements were solublized in water and drenched weekly. The calculated amount of Se delivered in the 0.3, 0.9 and 1.5 mg/kg weekly drench was 4.9, 14.7, and 24.5 mg Se per dose. Each composited drench was submitted for Se analysis (Center for Nutrition, Diagnostic Center for Population and Animal Health, Michigan State University, E. Lansing, MI) to verify the desired solution concentration. The 0.3 mg/kg weekly drench of inorganic sodium selenate was 82.5% higher (8.95 mg per dose) than targeted concentrations. The 0.3, 0.9 and 1.5 mg/kg weekly drenches of sodium selenite (4.85, 14.85, and 24.6 mg per dose, respectively) and organic SeY (1959 µg/g) were found to be within expected analytical variance of their targeted concentrations.

4.3.3 Se Administration

Selenium treatments were administered individually by oral drenching once weekly at an amount equal to the summed daily intake. Non Se-supplemented ewes

received water. The Se dose was suspended in a reasonable volume of water (5 mL for inorganic Se; more water was needed for the organic solutions, i.e., 11, 30 and 48 mL for 0.3, 0.9, and 1.5 mg/kg solutions, respectively), made up fresh each week, and administered with a dose syringe as sheep moved through a cutting chute. Color coding of Se sources to match ewe ear tags was utilized to maintain dosing accuracy. Sheep were treated once weekly for a total of 52 weeks. Lambs did not receive any additional Se supplementation after birth.

4.3.4   Sample Collection from Ewes and Lambs

Jugular venous blood was collected directly from all ewes every 3 months, and in 16 of 30 ewes of the no Se and 0.3 mg/kg dose groups every month, starting at study initiation. Immediately after parturition and before lambs had nursed, jugular venous blood was collected from lambs. Lamb blood was collected again at 48 hours postnatal. For whole-blood analysis, blood was collected into evacuated EDTA tubes (2 mL; final EDTA concentration 2 g/L; Becton Dickinson, Franklin Lakes, NJ) and stored on ice until it could be frozen at -20 °C. For serum analysis, blood was collected into evacuated tubes without EDTA (10 mL; Becton Dickinson). Tubes were centrifuged at 850 x g for 10 min; serum was collected, transferred into 2.0 mL screw cap self-standing micro tubes (ISC BioExpress, Kaysville, UT) and stored at -20 °C. Colostrum samples were collected immediately following parturition. Ewes were milked by hand and samples were collected into 15 mL centrifuge tubes (10 mL; ISC BioExpress, Kaysville, UT) and stored at -20 °C.

4.3.5   Se Analysis of Ewe and Lamb Samples

Selenium concentrations in whole blood, serum, and colostrum were determined by a commercial laboratory (Center for Nutrition, Diagnostic Center for Population and Animal Health, Michigan State University, E. Lansing, MI) using an ICP-MS method (Whalen et al., 2005) with modifications. Two-hundred μL of each whole-blood or serum sample was diluted 1:20 with a solution containing 0.5% EDTA and Triton X-100, 1% ammonia hydroxide, 2% propanol and 20 ppb of scandium, rhodium, indium and bismuth as internal standards. All samples were analyzed on an Agilent 7500ce ionized coupled plasma mass spectrometer. Selenium, at mass 78, was analyzed in hydrogen mode to reduce spectral interference.

4.3.7   Colostrum and Serum IgG Measurements

IgG concentrations in ewe colostrum and lamb serum were quantified using a direct ELISA procedure. The protocol was adapted from a commercial test not yet released by Bethyl Labs (Montgomery, TX). In brief, 96-well plates (Reacti-Bind™ Thermo Scientific, Rockford, IL) were coated with 100 μL of affinity purified rabbit anti-sheep heavy and light chain coating antibody (1 mg/mL; Bethyl Labs, Montgomery, TX) and incubated for 1 hour. The coating solution contained 0.05 M carbonate-bicarbonate buffer, pH 9.6.

After incubation, plates were aspirated and washed 3 times with T-PBS (50 nM Tris, 0.14 M NaCl, 0.05% Tween 20, pH 8.0). A Nunc-Immuno™ 8 and 12 channel plate washer (Thermofisher, Rockford, IL) was used for every washing step to maintain consistency from plate to plate. Plates were then blocked with Tris-PBS and incubated for 30 min. After incubation, the plates were washed three times in Tris-PBS.

Standards and samples were prepared by the following dilution schemes. Purified sheep IgG (Sigma, St Louis, MO) at 10 mg/mL served as the assay standard. It was diluted in Tris-PBS in duplicate for a standard curve ranging from 7 ng to 500 ng. Pre-suckle (0 hr) serum samples were diluted 1:100 and 1:500, whereas post-suckle (48 hr) serum samples were diluted 1:250,000, 1:500,000, and 1:1,000,000. Colostrum samples were diluted 1:800,000, 1:1,600,000, and 1:3,200,000. All sample dilutions were prepared in Tris-PBS. A multi-channel pipette (Labpette™, Woodbridge, NJ) was used to prepare all sample dilutions and for plating in order to maintain consistency from plate to plate. High and low IgG controls from two different sheep were included on every plate. All standards, samples, and controls were plated in duplicate at 100 μL per well, and allowed to incubate for 1 hour at room temperature.

After incubation, samples were aspirated and washed 5 times in Tris-PBS and then rabbit anti-sheep heavy and light chain coating antibody plus horse-radish peroxidase (HRP) conjugate (1 mg/mL; Bethyl Labs, Montgomery, TX) was added to the wells at a 1:500,000 dilution, and allowed to incubate for 1 hour at room temperature. After incubation, HRP-detection antibody was removed by washing each well 5 times in Tris-PBS.

After the final wash, 100 uL of tetramethyl-benzidine (TMB) (Sigma, St Louis, MO) was added to each well, and the plate was read immediately on an Ultramark Microplate Reader™ (Bio-Rad, Hercules, CA) at 655 nm in two-minute intervals until an absorbance of 0.650 to 0.800 O.D. was achieved in the 500 ng IgG standard well. At this point the TMB reaction was stopped with 100 μL of 2M H_2SO_4 and the plate was read at 450 nm.

4.3.8   Statistical Analysis

Statistical analyses were performed using SAS, version 9.1 (SAS, Inc., Cary, NC, USA) software. We only used data from ewes with both colostral and lamb IgG concentrations in the statistical analysis (no Se = 15 ewes; 0.3 mg/kg sodium selenate = 13 ewes; 0.3 mg/kg sodium selenite = 13 ewes; 0.9 mg/kg sodium selenite = 17 ewes; 1.5 mg/kg sodium selenite = 14 ewes; 0.3 mg/kg SeY = 17 ewes; 0.9 mg/kg SeY = 14 ewes; 1.5 mg/kg SeY = 10 ewes). Values from lambs of the same ewe were averaged because ewe was the experimental unit. The effect of source and amount of Se supplement (no Se, 0.3 mg/kg sodium selenate, 0.3 mg/kg sodium selenite, 0.9 mg/kg sodium selenite, 1.5 mg/kg sodium selenite, 0.3 mg/kg SeY, 0.9 mg/kg SeY, 1.5 mg/kg SeY) on IgG concentrations in ewe colostrum and serum of 2-day old lambs; whole-blood and serum-Se concentrations in ewes and lambs; and colostral-Se concentrations in ewes were analyzed using PROC GLM. Covariates in the model were FR-status (yes, no), breed (Polypay, Suffolk or crossbred), birth weight (<14 lbs, ≥ 14 lbs; values of lambs of the same ewe were averaged), number of lambs nursed (1, >1; we had insufficient numbers of ewes that gave birth to one lamb to do statistical analysis), and the interaction between birth weight and group. To evaluate whether FR-status, breed, and number of lambs nursed (1, >1) modified the effect of Se source and amount on IgG or Se concentrations, data were additionally stratified by FR-status, breed, birth weight, and number of lambs nursed, respectively.

The effect of no Se supplementation on IgG and Se concentrations was evaluated by comparing the estimated values of the no-Se group with those at the 0.3 mg/kg level. The effect of Se source was evaluated by comparing the estimated values of different Se

sources at the same Se dosage. The effect of Se amount was evaluated by comparing the estimated values of different Se dosages within the same Se source. Data are reported as least square means ± SEM. Statistical significance was declared at $P \leq 0.05$.

4.4 RESULTS

4.4.1 Colostral IgG Concentrations

Colostral IgG concentrations from ewes in all treatment groups are shown in **Table 1** and **Figure 1**. Ewes receiving Se doses of SeY or sodium selenite at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg did not have greater colostral IgG concentrations ($P = 0.82$) than ewes not supplemented with Se. Ewes receiving supranutritional Se doses of SeY greater than 0.3 mg/kg (i.e., 0.9 mg/kg and 1.5 mg/kg) did not have greater colostral IgG concentrations than ewes supplemented at the recommended 0.3 mg/kg SeY concentration ($P = 0.66$) and ($P = 0.31$), respectively. Ewes receiving supranutritional Se doses of sodium selenite greater than 0.3 mg/kg (i.e., 0.9 mg/kg and 1.5 mg/kg) did not have greater colostral IgG concentrations than ewes supplemented at the recommended 0.3 mg/kg sodium selenite concentration ($P = 0.12$) and ($P = 0.08$), respectively. Ewes receiving Se as SeY at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg did not have greater colostral IgG concentrations than ewes supplemented with sodium selenite at the corresponding 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg concentrations ($P = 0.23$). Finally, when comparing the colostral IgG concentrations of ewes supplemented with 0.3 mg/kg of sodium selenate or sodium selenite, there were no differences detected ($P = 0.20$).

Table 1. Whole-blood and colostral-Se concentrations and the IgG concentration of colostrum (least squared mean ± SEM) for ewes dosed weekly with inorganic or organic Se sources at varying concentrations, and the corresponding whole-blood and serum-Se concentrations, and 48 hr serum-IgG concentration of their lambs.

Se Source	Se dose, (mg/kg)	N	Whole Blood Se[1] (Early Lactation) (ng/mL)	Colostrum Se (At Parturition) (ng/mL)	Colostrum IgG (At Parturition) (mg/mL)	N	Whole Blood Se (At Birth) (ng/mL)	Serum Se (At Birth) (ng/mL)	Serum IgG (48 h Postnatal) (mg/ml)
			Ewes				Lambs		
No Se	0	15	112.8 ± 13.1^f	46.5 ± 36.9^f	66.8 ± 9.2^{ab}	15	102.3 ± 20.9^g	35.5 ± 4.3^g	28.6 ± 2.7^a
Selenate	0.3	13	337.9 ± 14.4^d	284.2 ± 40.6^{de}	68.4 ± 10.1^{ab}	13	264.2 ± 23.0^{ef}	66.3 ± 4.2^{ef}	28.9 ± 3.0^a
Selenite	0.3	13	289.5 ± 14.5^{de}	186.9 ± 41.0^e	51.0 ± 10.2^b	13	224.9 ± 23.2^f	58.8 ± 4.5^f	31.4 ± 3.0^a
	0.9	17	365.3 ± 12.3^{cd}	325.7 ± 35.7^{cd}	70.6 ± 8.7^{ab}	17	299.2 ± 19.7^{de}	77.1 ± 4.4^{de}	33.6 ± 2.6^a
	1.5	14	392.9 ± 14.1^c	476.4 ± 40.9^b	74.3 ± 9.9^{ab}	14	339.1 ± 22.5^d	84.6 ± 4.4^d	26.8 ± 2.9^a
Se Yeast	0.3	17	378.9 ± 12.2^c	403.7 ± 36.4^{bc}	66.4 ± 8.6^{ab}	17	403.9 ± 19.4^c	98.3 ± 4.2^c	27.4 ± 2.5^a
	0.9	14	528.4 ± 13.3^b	399.8 ± 38.6^{bc}	71.9 ± 9.4^{ab}	14	582.6 ± 21.2^b	125.9 ± 4.2^b	29.7 ± 2.8^a
	1.5	10	670.4 ± 15.8^a	1071.3 ± 44.6^a	80.4 ± 11.1^a	10	649.0 ± 25.3^a	182.6 ± 4.6^a	32.3 ± 3.3^a

[a,b,c,d,e,f,g,h] Within a column, means without a common superscript differ ($P < 0.05$).

[1] Ewe whole-blood Se samples were collected at approximately 2 weeks into lactation.

Figure 1. Colostral IgG concentrations (mg/mL) at parturition (least squared means ± SEM) from ewes in all Se-treatment groups.

Figure 1. Colostral IgG concentrations (mg/mL) at parturition (least squared means ± SEM) from ewes in all Se-treatment groups.
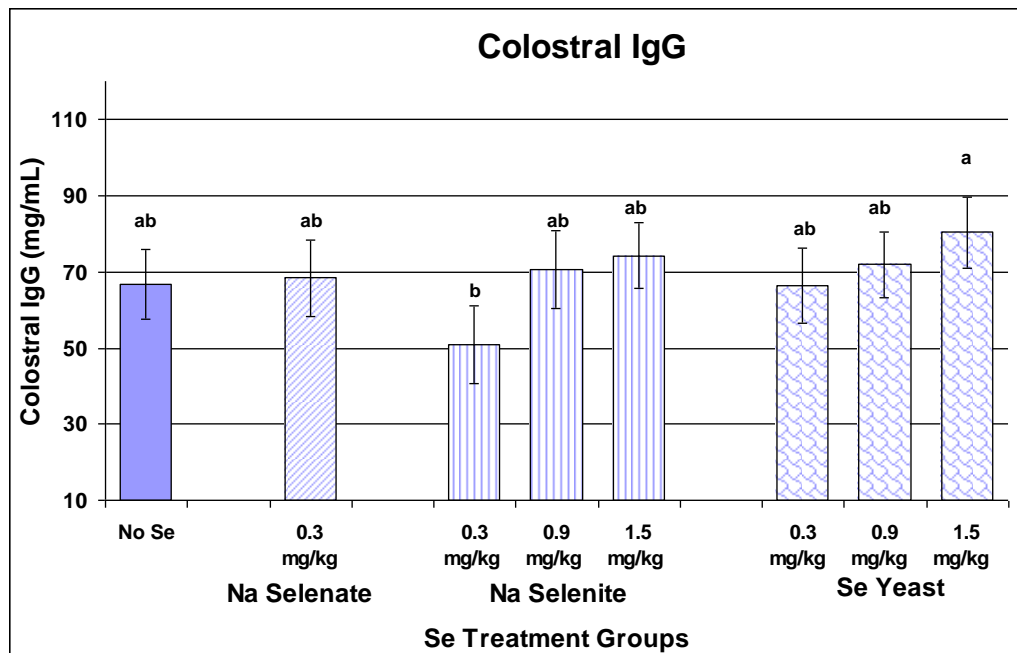
4.4.2   Lamb Serum IgG Concentrations Measured at 48 Hours

A comparison of lamb serum IgG concentrations are shown in **Table 1** and **Figure 2**. Lambs born from ewes receiving SeY or sodium selenite at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg did not have greater serum IgG concentrations at 48 hours than lambs from ewes not receiving supplemental Se ($P = 0.61$). Lambs from ewes receiving supranutritional Se doses of SeY greater than 0.3 mg/kg (i.e., 0.9 mg/kg and 1.5 mg/kg) did not have greater serum IgG concentrations at 48 hours than lambs from ewes supplemented at the recommended 0.3 mg/kg SeY concentration ($P = 0.52$) and ($P = 0.22$), respectively. Lambs from ewes receiving supranutritional Se doses of sodium selenite greater than 0.3 mg/kg (i.e., 0.9 mg/kg and 1.5 mg/kg) did not have greater serum IgG concentrations at 48 hours than lambs from ewes supplemented at the recommended

0.3 mg/kg sodium selenite concentration ($P = 0.56$) and ($P = 0.24$), respectively. Lambs from ewes receiving Se as SeY at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg did not have greater serum IgG concentrations at 48 hours than lambs from ewes supplemented with sodium selenite at the corresponding 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg concentrations ($P = 0.20$). Finally, when comparing the 48 hour serum IgG concentrations of lambs from ewes supplemented with 0.3 mg/kg of sodium selenate or sodium selenite, there were no differences detected ($P = 0.52$).

Figure 2. Lamb serum IgG concentrations (mg/mL) at 48 hours (least squared means ± SEM) born from ewes in all Se-treatment groups.
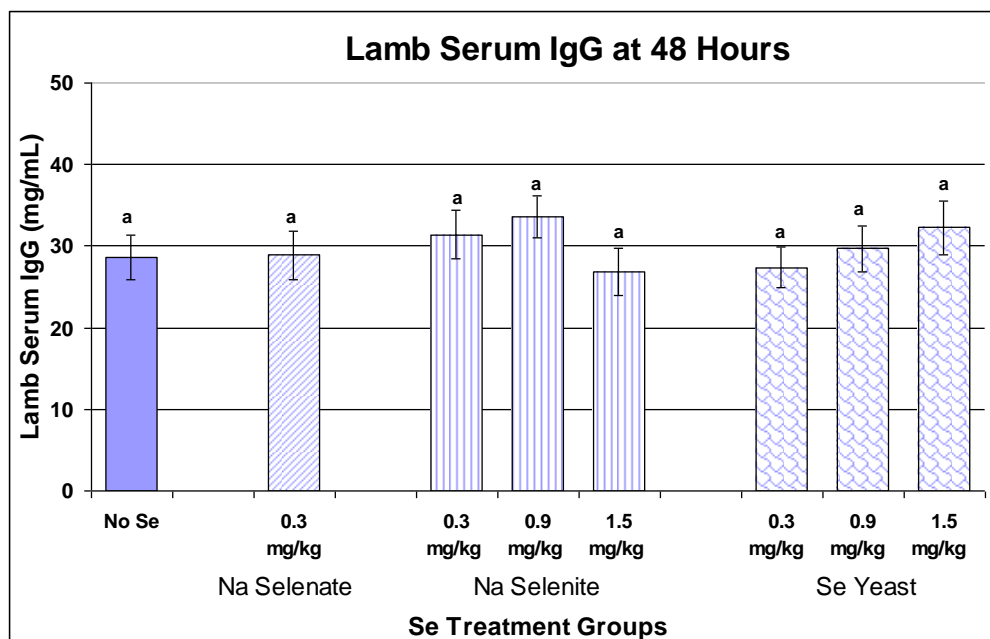
4.4.3 IgG Absorption Efficiency Ratios

The IgG absorption efficiency ratios were calculated for lambs from ewes in each Se-treatment group to compare how efficiently colostral IgG was transferred to and absorbed by the newborn lamb. The IgG absorption efficiency ratio is calculated by dividing the lamb serum IgG concentration by the ewe colostral IgG concentration. These IgG absorption efficiency ratios are shown in **Figure 3**. The only differences observed

between treatment groups were for lambs in the 0.3 mg/kg sodium selenite group. These
lambs had greater transfer efficiency ratios compared to lambs from all other treatment
groups ($P < 0.05$).

Figure 3. Lamb IgG absorption efficiency ratio (least squared mean ± SEM) showing 48
hour lamb serum IgG concentrations divided by ewe colostral IgG concentrations.
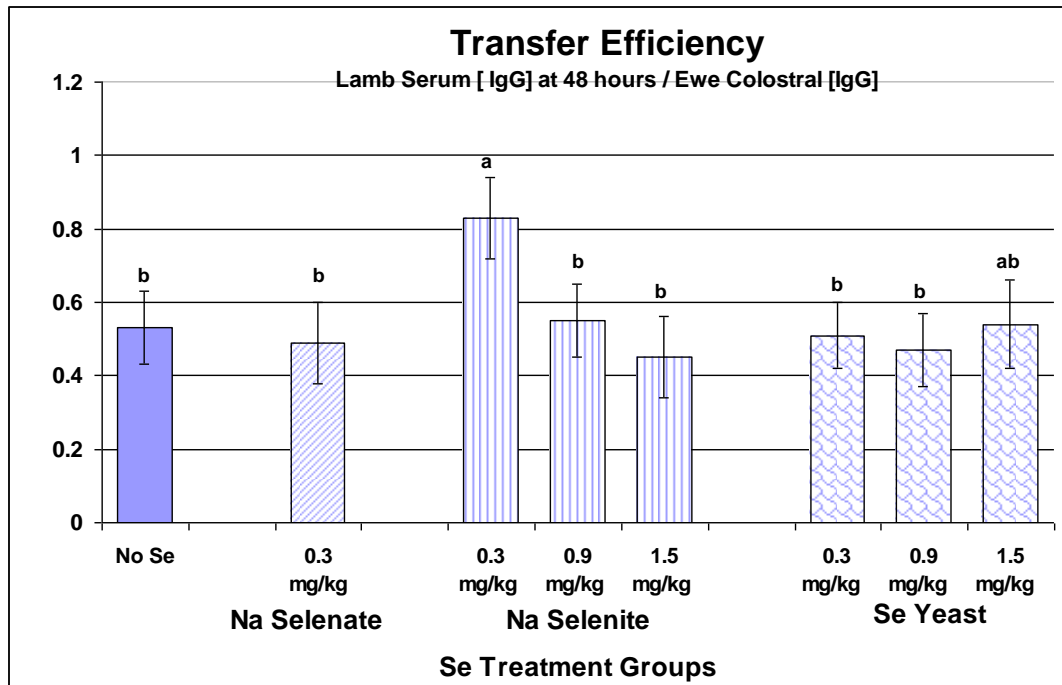
4.5 DISCUSSION AND CONCLUSION

4.5.1 Colostral IgG Concentrations

Colostral IgG concentrations in our study are numerically consistent to those in a previous study conducted with Oregon State University ewes of similar genetic origin (Al-Sabbagh et al., 1995). We did not observe significant differences when comparing colostral IgG concentrations in ewes from treatment groups receiving Se vs. groups not receiving Se. The highest colostral IgG concentrations in ewes across Se-treatment groups was for the 1.5 mg/kg SeY group, which had 47% higher colostral IgG concentrations compared to the 0.3 mg/kg sodium selenite group. The means for colostral IgG concentrations were numerically greater in ewes from treatment groups supplemented with Se at the 0.9 and 1.5 mg/kg concentrations compared to ewes receiving no Se or 0.3 mg/kg ($P > 0.05$). Our results agree with Rock et al. (2001) where no differences in colostral IgG concentrations were observed when comparing non-Se supplemented ewes and those supplemented with selenium yeast or sodium selenite at the recommended 0.3 mg/kg concentrations. Our findings are also consistent with Swanson et al. (2008) who found that colostral IgG concentrations in ewes receiving 0.1 mg/kg and 3.5 mg/kg SeY were unaffected by Se supplementation.

During the last 12 days of gestation, serum Ig concentrations in ewes begins to decrease as large amounts of Ig are transferred to the mammary gland (Mayer et al., 2002; Rodinova et al., 2008). Recently, it was shown that the same neonatal Fc receptor for IgG (FcRn) involved in intestinal absorption of Ig also plays an important role in IgG transport from ewe serum into colostrum (Mayer et al., 2002). Therefore, the IgG

concentration of colostrum is primarily a function of immunological history of the ewe and the efficiency of IgG transfer from serum to colostrum (Tizard, 2009). It is possible that Se may influence the expression of FcRn receptors in mammary tissue and, thus, facilitate the accumulation of IgG in colostrum. Future investigations involving this possible mechanism are warranted.

4.5.2   Lamb IgG Concentrations in 48 Hour Serum

The IgG concentrations in lamb serum measured in our study are consistent with reference ranges established in previous studies (Gilbert et al., 1988; Hunter et al., 1977). Failure of passive transfer can be influenced by multiple factors such as poor colostral quality, inadequate colostral ingestion, or intestinal absorption failure. McGuire et al. (1983) found that failure of passive transfer in neonatal lambs resulted in 45% dying before 3 weeks of age, whereas only 5% of the lambs with adequate passive transfer died. Similarly, we observed that lambs with serum IgG concentrations less than 15 mg/ml did not survive to weaning age (not reported). Thus, we agree with others who consider lamb serum-IgG concentrations to be an important measure influencing lamb survival. It is possible that confounding variables, such as variation in the amount of colostrum consumed and/or lamb hypothermia interfering with intestinal Ig absorption, precluded determination of whether IgG absorption was effected by Se source and supplementation rate in our study.

The lack of significant differences for serum-IgG concentrations in lambs from ewes receiving no Se supplement compared to lambs from all other Se-supplemented ewes may be attributed to a lack of Se deficiency status in ewes not receiving Se

supplement. Grace at al. (2002) suggested that noticeable Se-responsive improvement in animal performance is not normally observed unless Se concentrations of whole blood are 20 ng/mL or less. Thus, with mean whole-blood Se concentrations of 99 ng/mL in lambs from ewes receiving no Se supplement, Se concentrations were probably not low enough to negatively impact intestinal IgG absorption and, thus, alter serum-IgG concentrations.

Others have studied the effect of organic or inorganic Se supplementation in ewes on lamb IgG absorption (Rock et al., 2001; Rodinova et al., 2008). Rock et al. (2001) fed 0.3 mg/kg of SeY or sodium selenite, whereas Rodinova et al. (2008) fed 1.8 mg/kg of SeY or sodium selenite. Interestingly, both Rock et al. (2001) and Rodinova et al. (2008) observed significant increases in lamb serum-IgG concentrations measured at 12 hours (P <0.002; Rock et al., 2001) and 24 hours (P <0.05; Rodinova et al., 2008) in lambs from ewes receiving sodium selenite. It is also possible that ensuring an equivalent amount of colostrum was consumed by all lambs contributed to the significant findings in the Rock et al. (2001) and Rodinova et al. (2008) studies. It is also possible that the Se supplementation rate in ewes is more important than the Se source. A numerical increase in our study in serum-IgG concentrations of lambs born to ewes receiving increasing supranutritional SeY supplementation rates may reflect a similar numerical trend noted by Rock et al. (2001) and Rodinova et al. (2008) in lambs from ewes receiving 0.3 mg/kg sodium selenite.

A review of the literature suggest that stimulation of intestinal IgG absorption is greater for inorganic Se, specifically sodium selenite (Burton et al., 1977; Kamada et al.,

2007; Rock et al., 2001; Rodinova et al., 2008). One explanation for the increased IgG absorption observed in other studies might be that Se stimulates the intestinal epithelium by enhancing pinocytosis of Ig molecules. Burton et al. (1977) observed that Se and IgG form a complex *in vitro* that results in a structural change in the IgG molecule. Kamada et al. (2007) observed that addition of sodium selenite to bovine colostrum at 3 mg/kg increased IgG absorption in newborn dairy calves by 42%. Interestingly, optimal IgG absorption was achieved when calves consumed 3.0 mg/kg of added Se in the colostrum, over the range of 0.2 to 5.0 mg/kg of added Se (Kamada et al., 2007). In our study, the transfer efficiency (lamb serum IgG concentration at 48 hours divided by ewe colostral IgG concentration) was greater in lambs from ewes supplemented with 0.3 mg/kg sodium selenite, and transfer efficiency declined with increasing Se dosages.

At high doses, selenite has been reported to act as a pro-oxidant compound *in vitro* (Tiwary et al., 2006). This could explain why Se transfer efficiency was optimal for lambs from ewes supplemented with 0.3 mg/kg sodium selenite in our study. In those lambs, colostral Se concentration was 187 ng/mL. Lambs consuming colostrum from ewes not supplemented with Se received colostrum with Se concentration of 46 ng/mL. Perhaps this colostral Se concentration was too low for optimal transfer efficiency. Lambs consuming colostrum from all other Se supplemented ewes received colostrum with Se concentrations ranging from 284 to 1071 ng/mL. Perhaps these colostral-Se concentrations were too high for optimal transfer efficiency.

It is worthwhile mentioning the consistent numerical increase in colostral and lamb serum IgG concentrations as SeY supplementation rate increased. It is possible that

stimulation of intestinal pinocytosi is caused by specific selenoproteins unique to colostrum in ewes consuming SeY. In agreement with results of Juniper et al. (2006), we found Se concentrations in milk and colostrum were increased the most by SeY. Although Juniper et al. (2006) found Se concentrations in milk and colostrum of cows were increased by SeY, only 25 to 33% of the increase in total Se could be accounted for by increases in SeMet. This would indicate that the increased Se in colostrum is not just SeMet, and suggests that other selenoproteins are contained in colostrum, which may have an effect on intestinal pinocytosis. Milk contains a number of endogenous antioxidant enzymes, including glutathione peroxidase and thioredoxin reductases (Juniper et al., 2006). Perhaps an increased antioxidant capacity reduces epithelial turnover time. For example, the intestinal epithelium containing the FcRn receptors may not be sloughed off as quickly in the presence of antioxidant seleno-enzymes. In conclusion, further investigations are needed to identify what Se-specific selenoproteins are involved (if any) in protecting the intestinal epithelium.

4.6 REFERENCES

See Chapter 7.

CHAPTER 5. EFFECTS OF SELENIUM SOURCE AND SUPPLEMENTATION RATE IN EWES ON EWE PRODUCTIVITY AND LAMB PERFORMANCE

5.1 ABSTRACT

In the United States, the FDA regulates Se supplementation to ruminant diets at a level of 0.3 mg/kg Se (as fed basis). Questions still exist regarding what chemical form of Se is the most bioavailable supplement and what are the best supplementation rates for optimal productivity. To evaluate the effects of Se source and supplementation rate on ewe reproductive performance and subsequent vitality and growth performance of lambs, 240 ewes were divided into 8 treatment groups and drenched weekly (at an amount equal to their summed daily intake) for one year, including during gestation and early lactation, with no Se (deficient); at recommended levels (0.3 mg/kg) with inorganic sodium selenite, sodium selenate, or organic Se-yeast; or at supranutritional levels (0.9 and 1.5 mg/kg) with sodium selenite or Se yeast. A total of 88 ewes continued the study into year two for an additional 28 weeks. Year two treatments included no Se (deficient) ewes (n = 25); ewes supplemented at recommended levels (0.3 mg/kg) with organic Se-Y (n = 20); ewes supplemented at supranutritional levels (0.9 and 1.5 mg/kg) with organic Se-Y (n = 18 and 27, respectively).

In year one, lambing percent; number of lambs born per ewe; lamb birth weights; 90- and 120-day BW and ADG; and pounds of lamb weaned per ewe were measured. In year two, percentage of ewes exhibiting estrus; percentage of ewes exhibiting estrus that lambed; percentage of ewes lambing; and number of lambs born per ewe were measured

to assess ewe reproductive performance. Year two lamb performance measures included birth weights; neonatal vigor; 10-, 20-, and 60-day BW and ADG; and percentage of lambs surviving to 60 days.

In year one, Se supplementation, regardless of supplementation rate, did not affect lambing percent, number of lambs born per ewe, lamb birth weights, 90-day BW and ADG, or pounds of lamb weaned per ewe. However, lambs from ewes in the 1.5 mg/kg SeY group had greater120-day BW ($P = 0.10$) and ADG ($P = 0.06$) than lambs from ewes in the 0.3 mg/kg group. This same trend was apparent in a subgroup of lambs from Suffolk ewes receiving Se at 1.5 mg/kg that had greater 120-day BW ($P = 0.07$) and ADG ($P = 0.09$) compared to lambs receiving SeY at 0.3 mg/kg. Similarly, a subgroup of lambs reared as twins from ewes receiving Se at 1.5 mg/kg had greater120-day BW ($P = 0.07$) and ADG ($P = 0.08$) than lambs reared as twins from ewes in the 0.3 mg/kg group.

In year two, there were two ewe reproductive performance measures that were negatively affected by Se supplementation. First, the percentage of ewes exhibiting estrus that lambed in the 0.3 mg/kg SeY group (75%) was lower compared to ewes receiving no Se supplement (100%; $P = 0.02$). Also, the percentage of ewes lambing was lower ($P < 0.04$) for ewes receiving 0.3 mg/kg SeY (75%) compared to ewes receiving no Se supplemental (96%). Se supplementation, regardless of source and supplementation rate did not affect percentage of ewes exhibiting estrus or number of lambs born per ewe. There were three lamb performance measures that were affected by Se supplementation. Lambs from ewes receiving Se at 1.5 mg/kg tended to have greater lamb vigor scores compared to lambs from ewes receiving SeY at 0.3 mg/kg ($P = 0.07$). Second, the 60-

day ADG was greater for lambs from ewes in the 1.5 mg/kg group compared to lambs from ewes in the no Se group ($P =0.07$). A subgroup of lambs from Suffolk ewes receiving Se at 1.5 mg/kg had greater 60-day BW ($P = 0.03$) and ADG ($P = 0.06$) compared to lambs receiving SeY at 0.3 mg/kg. Similarly, a subgroup of lambs reared as twins from ewes receiving Se at 1.5 mg/kg had greater 60-day BW ($P = 0.08$) and ADG ($P = 0.06$) than lambs reared as twins from ewes in the 0.3 mg/kg group. Third, there was an increased percentage of lambs surviving to 60 days in lambs from ewes in the 1.5 mg/kg group compared to lambs from ewes in the no Se group (86% vs. 64%, respectively; $P =0.04$). Se supplementation, regardless of source and supplementation rate, did not affect lamb birth weight nor 10- and 20-day BW and ADG.

We conclude that supranutritional Se supplementation of ewes, primarily with SeY, positively affects later lamb performance measures (i.e., 60- and 120-day BW and ADG), and that these effects may be more pronounced for lambs of heavier breeds (i.e., Suffolk) and lambs reared as twins.

5.2   INTRODUCTION

Selenium was discovered as an essential micronutrient in 1958. After its discovery, researchers focused initially on the quantity of dietary Se needed to prevent diseases associated with Se deficiency. Selenium-responsive diseases, such as nutritional myopathy and Se-responsive unthriftiness (SeRU), can be prevented in young ruminants when adequate Se is provided (Muth et al., 1958). The current FDA recommendation is 0.3 mg Se/kg DM (FDA, 1987). The range of adequate Se supplementation is narrow, as toxicity signs occur when lambs are injected with sodium selenite above 1 mg/kg or are

fed SeMet above 4 mg/kg of body weight (BW) (Tiwary et al., 2006). Current FDA

regulations limit the amount of dietary Se added to 0.7 mg per head per day or to 90 mg

Se/kg of free-choice mineral supplement (FDA, 1987). Higher Se dosages are considered

supranutritional. However, current recommendations do not account for the chemical

form of Se [e.g., sodium selenite, sodium selenate, or selenium yeast (SeY)] and its effect

on bioavailability, which may change with dosage. The bioavailability of a trace mineral

refers to the degree to which an ingested nutrient is absorbed and available to the body.

Besides the chemical form of Se, other factors that influence Se bioavailability in sheep

are diet composition, type of digestive system (e.g., ruminant, pre-ruminant), route of

administration (diet, drench, injection), and production stage (Surai, 2006).

Selenium sources can be classified into two categories: inorganic and organic.

The most common inorganic Se sources are sodium selenite and sodium selenate, which

are usually provided in mineral premixes or are injected. Organic Se sources are seleno-

amino acids [e.g., selenomethionine (SeMet) and selenocysteine (SeCys)], which are

found in SeY or in feeds grown on Se-rich soils. Organic Se sources are more

bioavailable than inorganic Se sources because sodium selenite and sodium selenate must

first be converted to hydrogen selenide (H_2Se) and then to selenophosphate ($HSePO_3^{-2}$)

before they can be utilized in selenoprotein synthesis. For example, $HSePO_3^{-2}$ reacts with

tRNA-bound serinyl residues to give SeCys-bound tRNA from which SeCys is inserted

co-translationally at loci encoded by specific UGA codons, to give selenoproteins

(Rayman et al., 2008). In contrast, when SeMet is consumed it can be tran-selenated to

SeCys (by analogy with the trans-sulfuration pathway). Seleno-cysteine is then converted

to H_2Se by SeCys ß-lyase. Alternatively, SeMet can be incorporated into protein in place of methionine; thereby providing a Se depot (Rayman et al., 2008).

Others have shown that ewe fertility is highly correlated to adequate Se status. Gabryszuk and Klewiec (2002) injected ewes with sodium selenite 4 weeks prior to breeding and again during the last 4 weeks of gestation, and showed a 32% increase in lambing percentage when compared to Se-deficient ewes. Koyuncu and Yerlikaya (2007) observed increased numbers of ewes exhibiting estrus, and higher pregnancy rates, lambing rates, and twining rates when ewes were given one sodium selenite injection prior to breeding. Kott et al. (1983) discovered that pre-weaning lamb survival was increased when ewes received 5ml of injectable sodium selenite monthly for one year. In that study, treated ewes weaned approximately 20% more lambs per ewe mated than did Se-deficient ewes. Although it is well established that Se supplementation of deficient ewes enhances ewe reproductive performance, the effects of Se source and supplementation rate on reproductive performance have not been investigated.

Provision of supranutritional Se to pregnant females is also thought to be an effective means of meeting Se requirements and enhancing newborn lamb performance. Reed et al. (2007) found that ewes receiving supranutritional levels of SeY at 4.41 mg/day had heavier fetal BW compared to ewes receiving SeY at below recommended levels (0.37 mg/day). Newborn lamb vitality is also enhanced with perinatal Se supplementation. Munoz et al (2008) found that ewes supplemented with SeY throughout pregnancy, compared with ewes supplemented only in the third trimester, had lambs with increased vigor scores and increased survival rates.

Numerous studies have shown that Se supplementation enhances growth rates in Se-deficient sheep (Kumar et al., 2009; McDonald., 1975; Sheppard et al., 1984). Studies involving Se supplementation of pregnant ewes have also consistently demonstrated enhanced lamb growth rates (Abd El-Ghany et al., 2008; Gabryszuk and Klewiec, 2002; Koyuncu and Yerlikaya, 2007; Munoz et al., 2008).

The objectives of the first year of this study were to examine the effect of Se source and supplementation rates in ewes on ewe productivity and lamb performance. It was hypothesized that lambs from Se supplemented ewes would have greater growth rates than lambs from ewes not supplemented with Se and that lambs from ewes supplemented with Se at supranutritional rates (0.9 mg/kg and 1.5 mg/kg) would have better growth performance than lambs from ewes supplemented at the recommended rate (0.3 mg/kg). We also hypothesized that lamb performance would be greater in lambs from ewes supplemented with organic SeY compared to lambs from ewes supplemented with inorganic sodium selenite at corresponding supplementation rates.

The objectives of the second year of this study were to continue our observations for the SeY treated ewes, adding additional parameters to monitor ewe reproduction and lamb growth performance.

5.3   MATERIALS AND METHODS

5.3.1   Animals and Study Design

Experimental procedures used in this study were approved by the Institutional Animal Care and Use Committees of Oregon State University. This was a prospective, placebo-controlled clinical trial of 12-months duration involving 240 mature ewes from

three genotypes (Polypay, Suffolk, and crossbred). Ewes ranged in age and BW from 2 to 6 yr, and 51 to 93 kg, respectively. The experiments were conducted at the Oregon State University Sheep Center, Corvallis, Oregon.

Ewes were randomly assigned to 8 treatment groups (n = 30 each) based on Se supplementation rate (0, 0.3, 0.9 and 1.5 mg/kg) and source [sodium selenite, sodium selenate (0.3 mg/kg only), and SeY]. Treatment groups were blocked for foot rot (FR) incidence and severity; breed; and age of ewe. The four dose levels (0, 0.3, 0.9, and 1.5 mg/kg) corresponded to no Se supplementation (0 mg/kg); 0.7 mg/d or 1x the FDA allowed supplementation rate (0.3 mg/kg); 2.1 mg/d or 3x the FDA allowed supplementation rate (0.9 mg/kg); and 3.5 mg/d or 5x the FDA allowed rate (1.5 mg/kg). All dosages were below the maximum tolerable level (5 mg/kg) for small ruminants (NRC, 2007).

5.3.2 Selenium Sources

Two inorganic Se sources were used: sodium selenite and sodium selenate, both from the same source (RETORTE Ulrich Scharrer GmbH, Röthenbach, Federal Republic of Germany). Sodium selenite was 456,000 mg/kg Se or 45.6% Se, and sodium selenate was 418,000 mg/kg Se or 41.8% Se (NRC, 2001). The organic Se source (SeY, Prince Se Yeast 2000, Prince Agri Products Inc., Quincy, IL) had a guaranteed analysis of 2,000 mg/kg of organically bound Se. Selenium supplements were solublized in water and drenched weekly. The calculated amount of Se delivered in the 0.3, 0.9 and 1.5 mg/kg weekly drench was 4.9, 14.7, and 24.5 mg Se per dose. Each composited drench was submitted for Se analysis (Center for Nutrition, Diagnostic Center for Population and

Animal Health, Michigan State University, E. Lansing, MI) to verify the desired solution concentration. The 0.3 mg/kg weekly drench of inorganic sodium selenate was 82.5% higher (8.95 mg per dose) than targeted concentrations. The 0.3, 0.9 and 1.5 mg/kg weekly drenches of sodium selenite (4.85, 14.85, and 24.6 mg per dose, respectively) and organic SeY (1959 μg/g) were found to be within expected analytical variance of their targeted concentrations.

5.3.3 Se Administration

Selenium treatments were administered individually by oral drenching once weekly at an amount equal to the summed daily intake. Non Se-supplemented ewes received water. The Se dose was suspended in a reasonable volume of water (5 mL for inorganic Se; more water was needed for the organic solutions, i.e., 11, 30 and 48 mL for 0.3, 0.9, and 1.5 mg/kg solutions, respectively), made up fresh each week, and administered with a dose syringe as sheep moved through a cutting chute. Color coding of Se sources to match ewe ear tags was utilized to maintain dosing accuracy. Sheep were treated once weekly for a total of 52 weeks. Lambs did not receive any additional Se supplementation after birth.

5.3.4 Forage and Dietary Se Assay

All feeds consumed throughout the trial period were analyzed to determine dietary Se contributions. Pasture forage samples were obtained using a systematic grid pattern with one sample generated from 25 subsamples to obtain samples for Se analysis. Mineral supplement, grass hay, alfalfa hay, alfalfa pellets, and corn were also submitted for Se analysis. All feed samples were submitted to a commercial laboratory (Center for

Nutrition, Diagnostic Center for Population and Animal Health, Michigan State University, E. Lansing, MI) for forage Se analysis by inductively coupled argon plasma emission spectroscopy (ICP-MS) (Whalen et al., 2005). All samples were analyzed on an Agilent 7500ce ionized coupled plasma mass spectrometer. Selenium, at mass 78, was analyzed in hydrogen mode to reduce spectral interference.

5.3.5   Year One Ewe Reproductive Performance Measures

Ewe reproductive performance was assessed in year one by calculating lambing percentage and recording lambs number of born per ewe. Lambing percentages were calculated based on number of ewes exposed to the ram. A lamb born per ewe measure was calculated based on how many lambs were born to each ewe. Additional information regarding length of parturition and level of assistance required were also recorded. Any additional ewe-health concerns were noted including whether mastitis, retained placenta, or prolapse occurred.

5.3.6   Year One Lamb Performance Measures

Lamb sex and birth weights were recorded at parturition. Lamb health records also noted health concerns such as hypothermia and scours. In addition to lamb birth weights, other lamb performance measures included 90- and 120-day BW and ADG; and pounds of lamb weaned per ewe. If insufficient milk production or mastitis was present, lambs were removed from the ewe. Lambs from ewes in all treatment groups were weighed on two more occasions at approximately 80 and 115 days of age. Weights were obtained using a calibrated scale platform and were then adjusted to 90- and 120-day standardized weights. ADG's were calculated from 0 to 90 days and from 0 to 120 days.

The 90-day pre-weaning weights, the 120-day weaning weights, ADG's, and pounds of lamb weaned per ewe were calculated using the American Sheep Industry Association (ASIA, 2002) formulas.

5.3.7 Year Two Ewe Reproductive Performance Measures

In year two, ewe reproductive performance was assessed by calculating percentage of ewes exhibiting estrus; percentage of ewes exhibiting estrus that lambed; percentage of ewes lambing; and by recording the number of lambs born per ewe. A total of 88 ewes continued the study into year two for an additional 28 weeks. Year two treatments included no Se (deficient) ewes (n = 25); ewes supplemented at recommended levels (0.3 mg/kg) with organic Se-Y (n = 20); ewes supplemented at supranutritional levels (0.9 and 1.5 mg/kg) with organic Se-Y (n = 18 and 27, respectively).

Ewes were exposed to a ram equipped with a marking harness for 35 days. Breeding marks were recorded once weekly at the time of Se drenching to record ewes in estrus, and later were used to determine the percentage of ewes marked that actually lambed. Lambs born per ewe exposed were calculated using the American Sheep Industry Association (ASIA, 2002) formulas.

At parturition ewes were assigned a lambing difficulty score from 1 to 7: 1= no assistance; 2= minimal assistance (easy pull); 3= head back (hard pull); 4= leg back (hard pull); 5= both legs back (hard pull); 6= backwards (hard pull); 7= other complications resulting in death of lamb. The number of lambs born to each ewe was recorded at parturition.

5.3.8 Year Two Lamb Performance Measures

Lambs sex and birth weights were recorded at parturition. Lambs were also given ID tags at birth. Each lamb's behavior was observed and recorded. Lambing vigor scores were assigned for specific time intervals (0 to15 minutes, and 15 to 30 minutes) based on the following criteria: 1= dead; 2= hypothermic (recorded rectal temperature); 3= weak and lethargic; does not attempt to hold up head or stand; 4= holds up head; 5= holds up head and attempts to stand; 6= stands for over twenty seconds; 7= vigorous (stands immediately and attempts to nurse).

In addition to birth weights and vigor scores, other lamb performance measures included 10-, 20-, and 60-day BW and ADG, and percentage of lambs surviving to 60 days. In addition to lambs being weighed at birth, lambs were also weighed at three later time points: at approximately 8-, 16-, and 54-days of age. Adjusted weights were calculated for 10-, 20-, and 60-days of age. ADG's were calculated from 0 to 20 days, and from 0 to 60 days. The 10-, 20-, and 60-day pre-weaning weights, and the ADGs were calculated using the American Sheep Industry Association (ASIA, 2002) formulas. Percentage of lambs surviving to 60 days was also recorded for each ewe treatment group and calculated from the number lambs nursing that survived to the last weigh period.

5.3.9 Statistical Analysis

Statistical analyses were performed using SAS, version 9.1 (SAS, Inc., Cary, NC, USA) software. Values of lambs of the same ewe were averaged because ewe was the experimental unit. For continuous data, the effect of source and dosage rates of Se

supplement (no Se, 0.3 mg/kg sodium selenate, 0.3 mg/kg sodium selenite, 0.9 mg/kg sodium selenite, 1.5 mg/kg sodium selenite, 0.3 mg/kg SeY, 0.9 mg/kg SeY, 1.5 mg/kg SeY) were evaluated using PROC GLM (single measures; birth weights) and PROC Mixed (repeated measures; later body weights; and growth rates). For multinomial data (number of lambs born, lambing difficulty, vigor scores), a multinomial distribution using the cumulative logistic link function in PROC GENMOD was used. Fisher's exact test was used for binomial data (lamb survival, estrus percentage, lambing percentage). Covariates in the multinomial and continuous data models were FR-status (yes, no; only available for year 1), breed (Polypay, Suffolk or crossbred), number of lambs born (1, >1). For repeated measure analysis, age at sampling and the interaction between of age at sampling and group were added as fixed effects. To evaluate whether FR-status (only for year 1 data), breed, number of lambs born, and number of lambs reared (1, >1) modified the effect of Se source and supplementation rates, data were additionally stratified by FR-status, breed, number of lambs born, and number of lambs reared, respectively. A completely unrestricted variance-covariance structure was used to account for repeated measures taken on individual ewes across time. To obtain the correct degrees of freedom, the KENWARDROGER option was invoked. The KENWARDROGER option consists of the Satterthwaite adjustment for degrees of freedom with a Kenward-Roger adjustment on standard errors, which can be used for repeated measures studies. Lamb survival data and reproductive measures were analyzed using Fisher's exact test.

The effect of no Se supplementation on blood Se was evaluated by comparing the estimated values of the no-Se group with those at the 0.3 mg/kg level. The effect of Se

source was evaluated by comparing the estimated values of different Se sources at the same Se dosage. The effect of Se amount was evaluated by comparing the estimated values of different Se dosages within the same Se source. Weight data are reported as least square means $\pm$ SEM; the remaining measures are reported as means $\pm$ SEM. Statistical significance was declared at $P \leq 0.10$ for weight measures and $P \leq 0.05$ for the remaining measures.

5.4   RESULTS

5.4.1   Year One Ewe Reproductive Performance Measures: Lambing Percentage, Number of Lambs Born Per Ewe, and Lamb Birth Weights

The lambing percentage, number of lambs born per ewe, and lamb birth weights for the various Se treatment groups are shown in **Table 1**. Values in **Table 1** represent comparisons for the overall lamb crop and for breed of ewe (i.e., Polypay or Suffolk). In summary, Se supplementation, irrespective of source (i.e., SeY, sodium selenite, sodium selenate) and/or concentration (i.e., 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg), had no significant effect on lambing percentages in Year 1.

The number of lambs born per ewe from ewes in the various Se treatment groups are also shown in **Table 1**. In summary, Se supplementation irrespective of source (i.e., SeY, sodium selenite, sodium selenate) and/or concentration (i.e., 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg) had no significant effect on number of lambs born per ewe. Ewes receiving Se as SeY or sodium selenite at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg did not have greater number of lambs born per ewe than ewes not receiving supplemental Se. Furthermore, ewes receiving supranutritional SeY doses greater than 0.3 mg/kg (i.e., 0.9

mg/kg and 1.5 mg/kg) did not have greater number of lambs born per ewe than ewes supplemented at 0.3 mg/kg. This same trend was observed with ewes receiving supranutritional sodium selenite doses greater than 0.3 mg/kg (i.e., 0.9 mg/kg and 1.5 mg/kg). They did not have greater number of lambs born per ewe than ewes supplemented at 0.3 mg/kg. Also, ewes receiving SeY at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg did not have greater number of lambs born per ewe than those receiving sodium selenite at the corresponding 0.3 mg/kg, 0.9 mg/kg and 1.5 mg/kg concentrations. Finally, ewes receiving inorganic selenite or selenate at 0.3 mg/kg had a similar number of lambs born per ewe.

The birth weights of lambs from ewes in the various Se treatment groups are shown in **Table 1**. In summary, Se supplementation irrespective of source (i.e., SeY, sodium selenite, sodium selenate) and/or concentration (i.e., 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg) had no significant effect on lamb birth weights. Lambs born from ewes receiving supranutritional Se (SeY or sodium selenite) at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg did not have higher birth weights than lambs from ewes not receiving Se. Furthermore, lambs born from ewes receiving supranutritional Se as SeY at 0.9 mg/kg and 1.5 mg/kg did not have higher birth weights than lambs from ewes receiving Se at 0.3 mg/kg. This same trend was observed in lambs born from ewes receiving supranutritional Se as sodium selenite at 0.9 mg/kg and 1.5 mg/kg. They did not have higher birth weights than lambs from ewes receiving Se at 0.3 mg/kg. Also, lambs born from ewes receiving Se as SeY at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg did not have higher birth weights than lambs from ewes receiving Se as sodium selenite at the corresponding 0.3

mg/kg, 0.9 mg/kg, and 1.5 mg/kg concentrations. Finally, lambs born to ewes receiving inorganic selenite or selenate at 0.3 mg/kg had similar birth weights.

Table 1. Year one ewe reproductive performance measures: lambing percent, number of lambs born per ewe, and lamb birth weights (least squared means ± SEM) from ewes dosed weekly with inorganic or organic Se sources at varying dietary concentrations.

Se Source	Se dose (mg/kg)	Lambing %	N	Birth Weight (pounds)	Lambs born per ewe	N	Birth Weight (pounds)	Lambs born per ewe	N	Birth Weight (pounds)	Lambs born per ewe
			All Lambs[1]			Polypay Lambs[2]			Suffolk Lambs[3]		
No Se	0	93[a]	27	13.9 ± 0.4[a]	1.73 ± 0.12[a]	17	13.1 ± 0.4[abc]	1.83 ± 0.12[a]	10	14.9 ± 0.7[ab]	1.58 ± 0.23[ab]
Selenate	0.3	96[a]	28	14.0 ± 0.3[a]	1.70 ± 0.11[a]	16	14.1 ± 0.4[a]	1.76 ± 0.14[a]	12	13.6 ± 0.6[bc]	1.62 ± 0.18[ab]
Selenite	0.3	87[a]	26	13.9 ± 0.4[a]	1.63 ± 0.14[a]	14	13.2 ± 0.5[abc]	1.56 ± 0.22[a]	12	14.5 ± 0.6[ab]	1.75 ± 0.13[ab]
	0.9	93[a]	26	13.9 ± 0.4[a]	1.80 ± 0.12[a]	14	13.6 ± 0.5[ab]	1.81 ± 0.19[a]	12	14.2 ± 0.6[ab]	1.79 ± 0.15[a]
	1.5	90[a]	27	13.4 ± 0.4[a]	1.83 ± 0.10[a]	16	12.7 ± 0.5[bc]	1.71 ± 0.14[a]	12	14.1 ± 0.6[ab]	2.00 ± 0.11[a]
Se Yeast	0.3	90[a]	27	12.1 ± 0.4[b]	1.57 ± 0.14[a]	13	12.0 ± 0.5[c]	1.54 ± 0.14[a]	14	12.4 ± 0.5[c]	1.59 ± 0.23[ab]
	0.9	93[a]	26	14.0 ± 0.4[a]	1.60 ± 0.12[a]	16	12.7 ± 0.4[abc]	1.53 ± 0.15[a]	10	15.8 ± 0.6[a]	1.69 ± 0.21[ab]
	1.5	83[a]	23	13.3 ± 0.4[a]	1.50 ± 0.16[a]	17	12.1 ± 0.4[c]	1.70 ± 0.19[a]	7	15.2 ± 0.7[ab]	1.10 ± 0.28[b]

[a,b,c,] Within a column, means without a common superscript differ ($P < 0.05$).
[1] Analysis comparing all lambs from ewes in all treatment groups.
[2] Analysis comparing lambs from Polypay ewes in all treatment groups.
[3] Analysis comparing lambs from Suffolk ewes in all treatment groups.

5.4.2 Year One Lamb Performance Measures: 90- and 120-day Body Weights and Average Daily Gains

The 90- and 120-day body weight values comparing the overall lamb crop, type of rearing (i.e., 1 or 2 lambs), and ewe breed (i.e., Polypay or Suffolk) are displayed in **Table 2a**. The average daily gain from 0 to 90 days and 0 to 120 days are shown in **Table 2b**. Lambs born from ewes receiving Se as SeY or sodium selenite at 0.3mg/kg, 0.9 mg/kg, and 1.5 mg/kg did not have higher averages for 90- and 120-day body weights than lambs from ewes not receiving Se ($P > 0.05$). Lambs born from ewes receiving supranutritional Se as SeY at 1.5 mg/kg did have greater 120-day body weights than lambs from ewes receiving SeY at 0.3 mg/kg ($P = 0.10$). See **Figure 1.** This trend was also reflected in lamb average daily gain (ADG) to 120 days, whereby lambs born from ewes receiving supranutritional Se as SeY at 1.5 mg/kg had higher ADG averages for 0 to 120 days than lambs from ewes receiving SeY at 0.3 mg/kg ($P = 0.06$). See **Figure 2** and **Table 2b**. When comparing 120-day body weights and ADG of lambs from Suffolk ewes receiving SeY at 1.5 mg/kg to the lambs from Suffolk ewes receiving SeY at 0.3 mg/kg, the 1.5 mg/kg group had greater 120-day body weights and ADG ($P = 0.07$ and $P = 0.09$, respectively). See **Figures 3** and **4**. In contrast to the SeY groups, lambs born from ewes receiving supranutritional Se as sodium selenite at 0.9 mg/kg and 1.5 mg/kg did not have higher averages for 90- and 120-day body weights and 90- and 120-day ADG than lambs from ewes receiving sodium selenite at 0.3 mg/kg. See **Tables 2a and 2b**. Lambs born from ewes receiving Se as SeY at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg did not have greater 90- and 120-day body weights or 90-and 120-day ADG than lambs from ewes

receiving Se as sodium selenite at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg. Finally, lambs born from ewes receiving inorganic selenite or selenate at 0.3 mg/kg had similar 90- and 120-day body weights and 90- and 120-day ADG.

Table 2a. Year one lamb performance measures: 90- and 120-day body weights (least squared means ± SEM) in lambs from ewes dosed weekly with inorganic or organic Se sources at varying dietary concentrations.

Se Source	Se dose (mg/kg)	N	All Lambs[1] 90 Day Weights (pounds)	120 Day Weights (pounds)	N	Reared as Singles[2] 90 Day Weights (pounds)	120 Day Weights (pounds)	N	Reared as Twins[3] 90 Day Weights (pounds)	120 Day Weights (pounds)	N	Polypay Lambs[4] 90 Day Weights (pounds)	120 Day Weights (pounds)	N	Suffolk Lambs[5] 90 Day Weights (pounds)	120 Day Weights (pounds)
No Se	0	27	73.5 ± 1.9 [a]	80.9 ± 2.2 [ab]	7	76.9 ± 5.2 [a]	85.4 ± 5.9 [a]	20	65.7 ± 1.7 [a]	72.2 ± 2.0 [ab]	17	71.5 ± 2.3 [ab]	77.5 ± 2.2 [ab]	10	75.6 ± 3.3 [a]	84.4 ± 4.6 [ab]
Selenate	0.3	28	73.6 ± 1.8 [a]	79.4 ± 2.2 [ab]	13	75.4 ± 3.9 [a]	79.0 ± 4.4 [a]	15	64.1 ± 2.0 [ab]	71.6 ± 2.3 [ab]	16	73.1 ± 2.4 [ab]	78.6 ± 2.2 [ab]	12	72.9 ± 2.9 [a]	78.9 ± 4.2 [b]
Selenite	0.3	26	72.6 ± 1.9 [a]	78.6 ± 2.3 [ab]	7	75.2 ± 5.2 [a]	81.3 ± 6.0 [a]	19	63.5 ± 1.8 [ab]	69.5 ± 2.1 [bc]	14	72.1 ± 2.6 [ab]	78.2 ± 2.5 [ab]	12	72.3 ± 2.9 [a]	78.4 ± 4.2 [b]
	0.9	26	72.0 ± 1.9 [a]	77.5 ± 2.3 [b]	8	75.2 ± 4.8 [a]	80.3 ± 5.5 [a]	18	61.8 ± 1.8 [ab]	67.4 ± 2.1 [bc]	14	68.6 ± 2.5 [a]	73.7 ± 2.4 [a]	12	74.5 ± 3.0 [a]	80.4 ± 4.2 [ab]
	1.5	28	75.7 ± 1.9 [a]	81.9 ± 2.2 [ab]	7	81.3 ± 5.3 [a]	88.1 ± 6.0 [a]	21	65.7 ± 1.7 [a]	71.8 ± 2.0 [ab]	16	74.7 ± 2.6 [b]	79.6 ± 2.3 [b]	12	75.0 ± 3.0 [a]	83.1 ± 4.2 [ab]
Se Yeast	0.3	27	71.9 ± 1.8 [a]	77.5 ± 2.2 [b]	14	74.3 ± 3.7 [a]	79.7 ± 4.2 [a]	13	62.9 ± 2.2 [ab]	68.6 ± 2.5 [bc]	13	69.9 ± 2.6 [ab]	74.7 ± 2.4 [ab]	14	73.0 ± 2.7 [a]	79.2 ± 3.5 [b]
	0.9	26	74.7 ± 1.5 [a]	79.0 ± 2.2 [ab]	15	80.8 ± 3.5 [a]	85.0 ± 4.0 [a]	11	60.0 ± 2.3 [b]	64.5 ± 2.7 [c]	16	72.8 ± 2.3 [ab]	75.0 ± 2.2 [ab]	10	75.9 ± 3.1 [a]	83.7 ± 4.5 [ab]
	1.5	23	74.0 ± 2.0 [a]	82.5 ± 2.4 [a]	10	76.9 ± 4.3 [a]	85.3 ± 5.0 [a]	13	66.3 ± 2.2 [a]	75.5 ± 2.5 [a]	17	71.2 ± 2.3 [ab]	78.7 ± 2.2 [ab]	7	78.2 ± 3.7 [a]	91.1 ± 5.5 [a]

[a,b,c,] Within a column, means without a common superscript differ ($P < 0.10$).
[1] Analysis comparing all lambs from ewes in all treatment groups.
[2] Analysis comparing lambs raised as singles from ewes in all treatment groups.
[3] Analysis comparing lambs raised as twins from ewes in all treatment groups.
[4] Analysis comparing lambs from Polypay ewes in all treatment groups.
[5] Analysis comparing lambs from Suffolk ewes in all treatment groups.

Table 2b. Year one lamb performance measures: Average Daily Gains (ADG) (least squared means ± SEM) in lambs from ewes dosed weekly with inorganic or organic Se sources at varying dietary concentrations.

Se Source	Se dose, (mg/kg)	All Lambs[1]			Reared as Singles[2]			Reared as Twins[3]			Polypay Lambs[4]			Suffolk Lambs[5]		
		N	90 Day ADG (grams)	120 Day ADG (grams)	N	90 Day ADG (grams)	120 Day ADG (grams)	N	90 Day ADG (grams)	120 Day ADG (grams)	N	90 Day ADG (grams)	120 Day ADG (grams)	N	90 Day ADG (grams)	120 Day ADG (grams)
No Se	0	27	366 ± 9^a	307 ± 9^{ab}	7	388 ± 26^a	324 ± 22^a	20	331 ± 9^a	273 ± 8^{ab}	17	352 ± 12^{ab}	290 ± 8^{ab}	10	382 ± 17^a	327 ± 18^{ab}
Selenate	0.3	28	366 ± 9^a	301 ± 8^{ab}	13	381 ± 15^a	301 ± 17^a	15	323 ± 10^{ab}	270 ± 9^{ab}	16	361 ± 12^{ab}	295 ± 8^{ab}	12	368 ± 15^a	303 ± 16^{ab}
Selenite	0.3	26	355 ± 10^a	298 ± 9^{ab}	7	377 ± 26^a	303 ± 23^a	19	320 ± 9^{ab}	264 ± 8^{bc}	14	350 ± 14^{ab}	292 ± 9^{ab}	12	365 ± 15^a	301 ± 16^b
	0.9	26	356 ± 10^a	294 ± 9^b	8	381 ± 24^a	306 ± 21^a	18	311 ± 9^{ab}	255 ± 8^{bc}	14	337 ± 13^a	276 ± 9^a	12	376 ± 15^a	312 ± 17^{ab}
	1.5	28	374 ± 9^a	311 ± 8^{ab}	7	405 ± 26^a	334 ± 23^a	21	331 ± 8^a	272 ± 7^{ab}	16	367 ± 13^b	258 ± 9^b	12	378 ± 15^a	322 ± 17^{ab}
Se Yeast	0.3	27	359 ± 9^a	293 ± 8^b	14	376 ± 18^a	304 ± 16^a	13	317 ± 11^a	258 ± 10^{bc}	13	350 ± 11^{ab}	282 ± 9^{ab}	14	368 ± 14^a	305 ± 15^b
	0.9	26	374 ± 9^a	300 ± 8^{ab}	15	408 ± 18^a	324 ± 15^a	11	302 ± 12^b	244 ± 10^c	16	363 ± 12^{ab}	282 ± 9^{ab}	10	383 ± 16^a	320 ± 17^{ab}
	1.5	23	370 ± 10^a	316 ± 9^a	10	388 ± 21^a	324 ± 15^a	13	334 ± 11^a	286 ± 10^a	17	353 ± 12^{ab}	295 ± 8^{ab}	7	394 ± 19^a	348 ± 21^a

[a,b,c,] Within a column, means without a common superscript differ ($P < 0.10$).
[1] Analysis comparing all lambs from ewes in all treatment groups.
[2] Analysis comparing lambs raised as singles from ewes in all treatment groups.
[3] Analysis comparing lambs raised as twins from ewes in all treatment groups.
[4] Analysis comparing lambs from Polypay ewes in all treatment groups.
[5] Analysis comparing lambs from Suffolk ewes in all treatment groups.

Figure 1. Year one lamb performance measures: 120-day body weights (least squared means ± SEM) in lambs from ewes dosed weekly with organic SeY at varying dietary concentrations. [a,b,c,d] Bars without a common superscript differ ($P < 0.10$).

Figure 2. Year one lamb performance measures: 120-day ADG (least squared means ± SEM) in lambs from ewes dosed weekly with organic SeY at varying dietary concentrations. [a,b,c,d] Bars without a common superscript differ ($P < 0.10$).

Figure 3. Year one lamb performance measures: 120-day body weights (least squared means ± SEM) in lambs from Suffolk ewes dosed weekly with organic SeY at varying dietary concentrations. [a,b,c,d] Bars without a common superscript differ ($P < 0.10$).
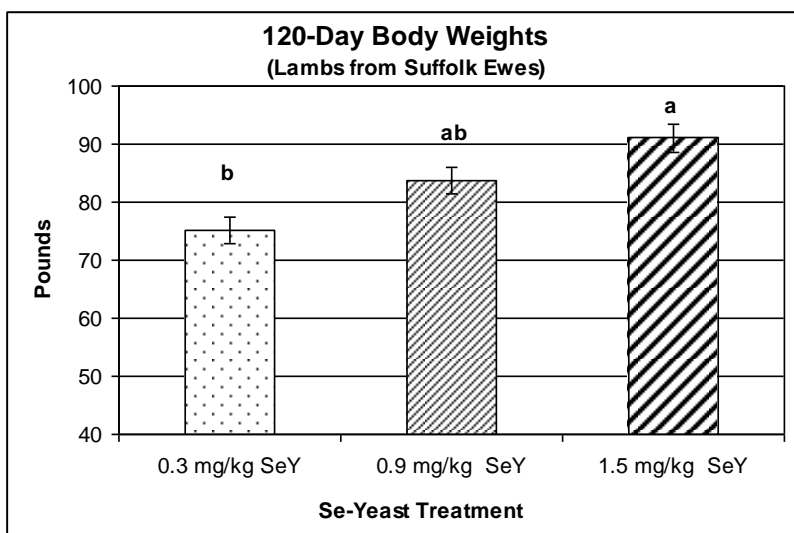
Figure 4. Year one lamb performance measures: 120-ADG (least squared means ± SEM) in lambs from Suffolk ewes dosed weekly with organic SeY at varying dietary concentrations. [a,b,] Bars without a common superscript differ ($P < 0.10$).
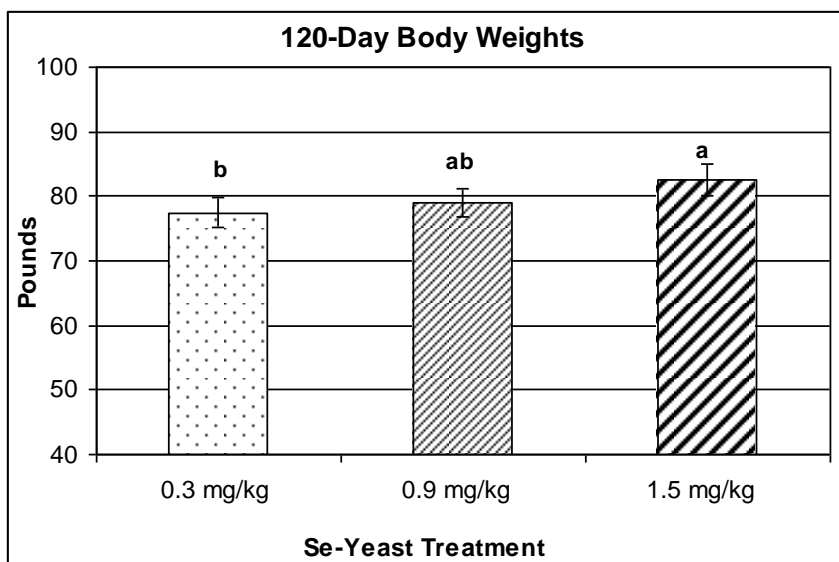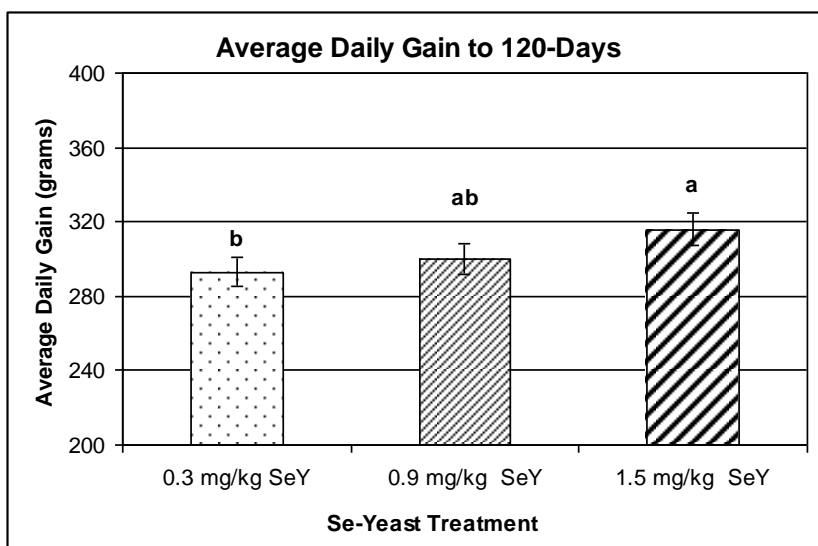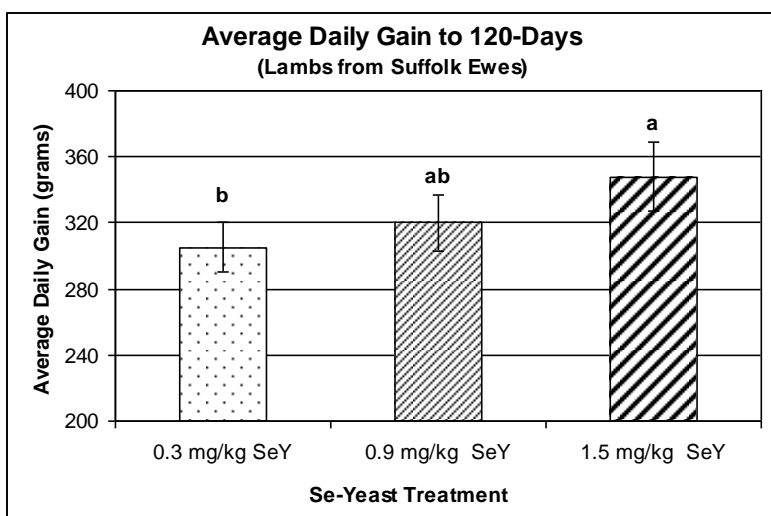
5.4.3   Year One Lamb Performance Measures: Pounds of Lamb Weaned Per Ewe

The pounds of lamb weaned per ewe values comparing the overall lamb crop, and types of rearing (i.e., 1 or 2 lambs) across treatment groups are displayed in **Table 3**. In summary, Se supplementation irrespective of source (i.e., SeY, sodium selenite, sodium selenate) and/or concentration (i.e., 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg) had no significant effect on the pounds of lamb weaned per ewe. Ewes receiving Se as SeY or sodium selenite at 0.3 mg/kg, 0.9 mg/kg and 1.5 mg/kg did not wean more pounds of lamb per ewe when comparing all lambs, lambs raised as singles, or lambs raised as twins, than ewes not receiving supplemental Se. Ewes receiving supranutritional SeY doses greater than 0.3 mg/kg (i.e., 0.9 mg/kg and 1.5 mg/kg) did not wean more pounds of lamb per ewe when comparing all lambs, lambs raised as singles, or lambs raised as twins, than ewes supplemented at 0.3 mg/kg. This same trend was observed in ewes receiving supranutritional sodium selenite in doses greater than 0.3 mg/kg (i.e., 0.9 mg/kg and 1.5 mg/kg). They did not wean more pounds of lamb per ewe when comparing all lambs, lambs raised as singles, or lambs raised as twins, than ewes supplemented at 0.3 mg/kg. Furthermore, ewes receiving SeY at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg did not wean more pounds of lamb per ewe when comparing all lambs, lambs raised as singles, or lambs raised as twins, than those receiving sodium selenite at the corresponding 0.3 mg/kg, 0.9 mg/kg and 1.5 mg/kg concentrations. Finally, ewes receiving inorganic selenite or selenate at 0.3 mg/kg had similar pounds of lamb weaned per ewe.

Table 3. Year one lamb performance measures: pounds of lamb weaned per ewe (least squared means $\pm$ SEM) from ewes dosed weekly with inorganic or organic Se sources at varying dietary concentrations.

Se Source	Se dose, (mg/kg)	N	All Ewes[1] Pounds Weaned Per Ewe	Reared as Single[2] Pounds Weaned Per Ewe	Reared as Twin[3] Pounds Weaned Per Ewe
No Se	0	27	107.1 ± 7.2^a	85.7 ± 6.0^a	144.5 ± 4.4^a
Selenate	0.3	28	103.3 ± 7.1^a	79.8 ± 4.4^a	143.6 ± 5.1^{ab}
Selenite	0.3	26	101.5 ± 7.2^a	80.2 ± 6.0^a	138.9 ± 4.6^{ab}
	0.9	26	99.2 ± 7.2^a	81.2 ± 6.6^a	134.7 ± 4.7^{ab}
	1.5	27	104.4 ± 7.1^a	88.2 ± 6.1^a	140.0 ± 4.3^{ab}
Se Yeast	0.3	27	101.2 ± 7.2^a	82.0 ± 4.5^a	136.6 ± 5.6^{ab}
	0.9	26	99.7 ± 7.2^a	86.2 ± 4.2^a	128.8 ± 6.0^b
	1.5	23	106.2 ± 6.7^a	85.1 ± 5.2^a	144.1 ± 5.6^{ab}

[a,b,] Within a column, means without a common superscript differ ($P < 0.10$).
[1] Analysis comparing all lambs from ewes in all treatment groups.
[2] Analysis comparing lambs raised as singles from ewes in all treatment groups.
[3] Analysis comparing lambs raised as twins from ewes in all treatment groups.

5.4.4 Year Two Ewe Reproductive Performance Measures

For year two, the reproductive performance measures: percentage of ewes exhibiting estrus; percentage of ewes exhibiting estrus that lambed; percentage of ewes lambing; and number of lambs born per ewe are summarized in **Table 4**. Compared to ewes receiving no Se, ewes receiving SeY at the 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg concentrations did not have greater estrus percentages (marked by ram). Compared to ewes receiving Se at 0.3mg/kg, ewes receiving SeY at the 0.9 mg/kg and 1.5 mg/kg concentrations did not have greater percentage of ewes exhibiting estrus ($P > 0.05$).

At lambing, compared to ewes receiving no Se, ewes receiving SeY at the 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg concentrations did not have a greater percentage of ewes exhibiting estrus that lambed. In contrast, the ewes receiving no Se had a significantly higher percentage of ewes exhibiting estrus that lambed (100%) when compared to ewes receiving SeY at 0.3 mg/kg (75%) ($P <0.007$). Furthermore, compared to ewes receiving Se at 0.3 mg/kg, ewes receiving SeY at the 0.9 mg/kg and 1.5 mg/kg concentrations did have a greater percentage of ewes marked by the ram that remained pregnant and gave birth, although not statistically significant ($P >0.05$)

For overall percentage of ewes lambing, compared to ewes receiving no Se, ewes receiving SeY at the 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg concentrations did not have a greater lambing percentage. Similar to the percentage of ewes exhibiting estrus that lambed, the ewes receiving no Se had a significantly higher percentage of ewes lambing when compared to ewes receiving SeY at 0.3 mg/kg ($P <0.04$). Compared to ewes receiving Se at 0.3mg/kg, ewes receiving SeY at the 0.9 mg/kg and 1.5 mg/kg concentrations did not have a greater percentage of ewes lambing.

Table 4. Year two ewe reproductive performance measures: percentage of ewes exhibiting estrus; percentage of ewes exhibiting estrus that lambed; and percentage of ewes lambing for ewes dosed weekly with inorganic or organic Se sources at varying dietary concentrations.

Year Two Ewe Reproductive Performance Measures					
Se Source	Se dose (mg/kg)	N	% Ewes Exhibiting Estrus	% Estrus that Lambed	% Ewes that Lambed
No Se	0	23	96^a	100^a	96^a
Se-Yeast	0.3	20	100^a	75^b	75^b
	0.9	18	100^a	89^{ab}	89^{ab}
	1.5	27	93^a	92^{ab}	85^{ab}

[a,b] Within a column, percentages without a common superscript differ ($P < 0.05$).

5.4.5   Year Two Ewe Reproductive Performance Measures: Number of Lambs Born Per Ewe and Lamb Birth Weights

The number of lambs born per ewe overall, and number of lambs born per ewe breed (i.e., Polypay, and Suffolk) are summarized in **Table 5**. The birth weights of all lambs and lambs according to ewe breed (i.e., Polypay, and Suffolk) are also summarized in **Table 5**. In summary, supranutritional SeY supplementation had no significant effect on number of lambs born per ewe and lamb birth weights. Compared to ewes receiving no Se, ewes receiving SeY at the 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg concentrations did not have a greater number of lambs born per ewe overall or according to ewe breed. Furthermore, compared to ewes receiving Se at 0.3 mg/kg, ewes receiving SeY at the 0.9 mg/kg and 1.5 mg/kg concentrations did not have a greater number of lambs born per ewe overall or according to ewe breed. Lamb birth weights also were not affected by Se supplementation. Compared to lambs from ewes receiving no Se, lambs born to ewes receiving SeY at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg concentrations did not have heavier birth weights. Similarly, compared to lambs from ewes receiving Se at 0.3 mg/kg, lambs born to ewes receiving SeY at 0.9 mg/kg and 1.5 mg/kg concentrations did not have heavier birth weights.

Table 5. Year two ewe reproductive performance measures: number of lambs born per ewe, and lamb birth weights (least squared means ± SEM) from ewes dosed weekly with an organic Se source at varying dietary concentrations.

Se Source	Se dose (mg/kg)	All Lambs[1]				Polypay Lambs[2]				Suffolk Lambs[3]		
		N	Birth Weight (pounds)	Lambs born per ewe		N	Birth Weight (pounds)	Lambs born per ewe		N	Birth Weight (pounds)	Lambs born per ewe
No Se	0	22	10.0 ± 0.5^a	1.57 ± 0.14^a		15	8.7 ± 0.5^a	1.56 ± 0.18^a		7	11.6 ± 1.0^a	1.57 ± 0.20^a
Se-Yeast	0.3	14	9.6 ± 0.6^a	1.35 ± 0.22^a		7	9.6 ± 0.7^a	1.44 ± 0.29^a		7	9.6 ± 1.1^a	1.27 ± 0.33^a
	0.9	16	10.1 ± 0.6^a	1.67 ± 0.18^a		12	8.7 ± 0.6^a	1.62 ± 0.18^a		4	12.2 ± 1.4^a	1.80 ± 0.49^a
	1.5	22	10.2 ± 0.5^a	1.55 ± 0.15^a		15	9.0 ± 0.5^a	1.67 ± 0.18^a		7	11.2 ± 1.0^a	1.33 ± 0.15^a

[a]Within a column, percentages without a common superscript differ ($P < 0.05$).
[1]Analysis comparing all lambs from ewes in all treatment groups.
[2]Analysis comparing lambs from Polypay ewes in all treatment groups.
[3]Analysis comparing lambs from Suffolk ewes in all treatment groups.

5.4.6 Year Two Lamb Performance Measures: Lambing Difficulty Scores and Lamb Vigor Scores

Lambing (birth) difficulty scores and lamb vigor scores overall, and according to ewe breed (i.e., Polypay and Suffolk) are displayed in **Table 6**. In summary, no effect of supranutritional SeY supplementation on birth difficulty scores was observed across treatment groups. No effect of Se on birth difficulty scores was observed when comparing ewes receiving no Se to ewes receiving Se at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg concentrations. Compared to ewes receiving Se at 0.3mg/kg, ewes receiving SeY at the 0.9 mg/kg and 1.5 mg/kg concentrations did not have lower lambing difficulty scores.

Lamb vigor scores tended to be influenced by supranutritional Se supplementation. Compared to lambs from ewes receiving no Se, lambs born to ewes receiving SeY at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg did not have greater lamb vigor scores. However, when comparing lambs from ewes receiving Se at 0.3 mg/kg, lambs born to ewes receiving SeY at 1.5 mg/kg concentrations did have numerically greater lamb vigor scores, although not statistically significant ($P = 0.07$). Also, lambs born to ewes receiving SeY at 1.5 mg/kg had greater lamb vigor scores when compared to lambs from ewes receiving SeY at 0.9 mg/kg ($P = 0.03$). See **Figure 6**.

Table 6. Year two lamb performance measures: birth (lambing) difficulty scores and lamb vigor scores (least squared means ± SEM) in lambs from ewes dosed weekly with an organic Se source at varying dietary concentrations. Comparisons are made for all lambs, lambs from Polypay ewes, and lambs from Suffolk ewes.

Se Source	Se dose (mg/kg)	All Lambs			Polypay Lambs			Suffolk Lambs		
		N	Birth[1] Difficulty Score	Lamb Vigor[2] Score	N	Birth[1] Difficulty Score	Lamb Vigor[2] Score	N	Birth[1] Difficulty Score	Lamb Vigor[2] Score
No Se	0	22	1.33 ± 0.17[a]	6.37 ± 0.17[a]	15	1.32 ± 0.19[a]	6.29 ± 0.23[a]	7	1.36 ± 0.36[a]	6.61 ± 0.15[a]
Se-Yeast	0.3	14	1.39 ± 0.27[a]	5.97 ± 0.21[ab]	7	1.43 ± 0.43[a]	5.94 ± 0.29[ab]	7	1.36 ± 0.36[a]	6.50 ± 0.24[a]
	0.9	16	1.60 ± 0.42[a]	5.90 ± 0.29[b]	12	1.23 ± 0.22[a]	5.86 ± 0.32[b]	4	2.63 ± 1.46[a]	6.00 ± 0.31[a]
	1.5	22	1.26 ± 0.26[a]	6.53 ± 0.16[a]	15	1.38 ± 0.38[a]	6.61 ± 0.15[a]	7	1.00 ± 0.00[a]	6.36 ± 0.42[a]

[ab]Within a column, means without a common superscript differ ($P < 0.05$).
[1]Birth (lambing) difficulty score criteria: 1= no assistance; 2= minimal assistance (easy pull); 3= head back (hard pull); 4= leg back (hard pull); 5= both legs back (hard pull); 6= backwards (hard pull); 7= other complications resulting in death of lamb.
[2]Lamb vigor score criteria: 1= dead; 2= hypothermic (recorded rectal temperature); 3= weak and lethargic; does not attempt to hold up head or stand; 4= holds up head; 5= holds up head and attempts to stand; 6= stands for over twenty seconds; 7= vigorous (stands immediately and attempts to nurse).

Figure 6. Year two lamb performance measures: lamb vigor scores (least squared means ± SEM) for all lambs. Lamb vigor score criteria: 1= dead; 2= hypothermic (recorded rectal temperature); 3= weak and lethargic; does not attempt to hold up head or stand; 4= holds up head; 5= holds up head and attempts to stand; 6= stands for over twenty seconds; 7= vigorous (stands immediately and attempts to nurse). [a,b] Bars without a common superscript differ ($P < 0.05$).
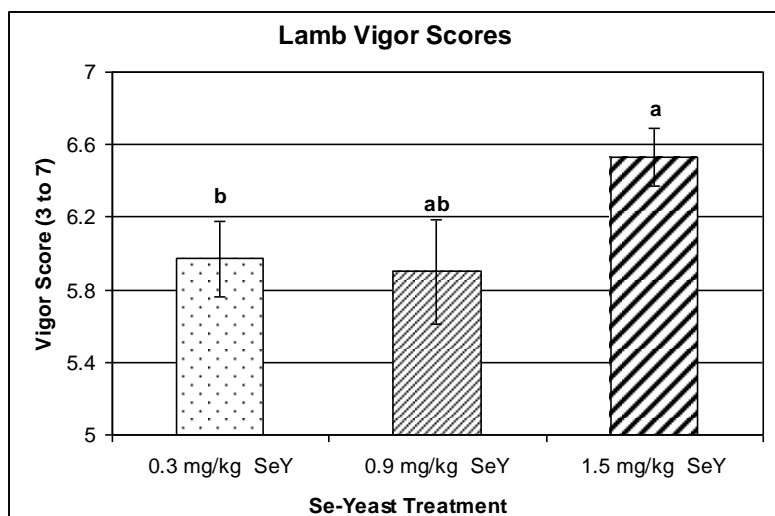
5.4.7 Year Two Lamb Performance Measures: 10-, 20-, and 60-Day Body Weights and ADG Measures

Lamb body weights at 10-, 20-, and 60-days are shown in **Table 7a**. Additionally, ADG values at 10-, 20-, and 60-days are displayed in **Table 7b**. Different comparisons for all lambs, for lambs according to number reared (i.e., 1 or 2 lambs), and for lambs according to ewe breed (i.e., Polypay or Suffolk) are also included for 10-, 20-, 60-day body weights and ADG in **Tables 7a and 7b**. Results for the overall lamb crop showed that when compared to lambs from ewes receiving no Se, lambs born to ewes receiving SeY at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg concentrations did not exhibit greater weights at 10-days, 20-days, or 60-days. Similarly, for the overall lamb crop, when comparing lambs from ewes receiving Se at 0.3 mg/kg, lambs born to ewes receiving SeY

at 0.9 mg/kg and 1.5 mg/kg concentrations did not exhibit greater weights at 10-days, 20-days, or 60-days.

In contrast, a breed specific supranutritional SeY effect on 10-, 20-, and 60-day body weights was observed as the age of the lamb increased when comparing lambs from Suffolk ewes across Se-treatment groups. Compared to lambs from Suffolk ewes receiving SeY at 0.3 mg/kg, lambs born to Suffolk ewes receiving SeY at 0.9 mg/kg began to have heavier weights at 10 days ($P =0.13$). This was more pronounced for 20-day body weights ($P =0.06$). Finally, at 60 days, body weights for both the 0.9 and 1.5 mg/kg SeY groups were significantly higher than body weights for lambs from ewes receiving 0.3 mg/kg SeY ($P =0.03$). See **Figures 7a and 7b**. This effect for supranutritional SeY was also observed when comparing lambs reared as twins across Se-treatment groups versus lambs reared as twins from ewes receiving no Se treatment. Lambs reared as twins from ewes receiving SeY at 1.5 mg/kg had heavier 60-day body weights than lambs born to ewes not receiving Se ($P =0.05$). See **Table 7**. Also, lambs reared as twins from ewes in the 0.9 mg/kg SeY group had heavier body weights at 60 days than lambs from ewes in the 0.3 mg/kg SeY group ($P =0.08$). See **Figures 8a and 8b.**

Lamb ADG showed similar trends to those observed for 10-, 20-, and 60-day body weights (**Figure 9**). When looking at the ADG for the overall lamb crop, compared to lambs from ewes receiving no Se, lambs born to ewes receiving SeY at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg concentrations did not exhibit greater ADG to 10 days or ADG to 20 days. However, lambs from ewes supplemented with SeY at 1.5 mg/kg tended to have

greater ADG to 60 days (P =0.07) compared to lambs from ewes receiving no Se (**Figure 9**). This same trend was statistically significant in the lambs being reared as twins, whereby compared to lambs from ewes receiving no Se, lambs born to ewes receiving SeY at 0.3 mg/kg, 0.9 mg/kg, and 1.5 mg/kg concentrations had greater ADG to 60 days (P <0.05). See **Figure 10**.

Furthermore, lambs being reared as twins from ewes receiving SeY at 0.9 mg/kg and 1.5 mg/kg had greater ADG to 60 days than lambs being reared as twins from the no Se group (P =0.03) or the 0.3 mg/kg SeY group (P =0.06). See **Figure 10**.

Finally, compared to lambs from Suffolk ewes receiving Se at 0.3 mg/kg, lambs born from Suffolk ewes receiving SeY at 0.9 mg/kg and 1.5 mg/kg concentrations had greater ADG to 60 days (P =0.06). See **Figure 11**.

Table 7a. Year two lamb performance measures: 10-, 20-, 60-day body weights (least squared means ± SEM) in lambs from ewes dosed weekly with an organic Se source at varying dietary concentrations.

Se Source	Se dose (mg/kg)	N	All Lambs[1] 10 Day Weight (pounds)	20 Day Weight (pounds)	60 Day Weight (pounds)	N	Reared as Singles[2] 10 Day Weight (pounds)	20 Day Weight (pounds)	60 Day Weight (pounds)	N	Reared as Twins[3] 10 Day Weight (pounds)	20 Day Weight (pounds)	60 Day Weight (pounds)	N	Polypay Lambs[4] 10 Day Weight (pounds)	20 Day Weight (pounds)	60 Day Weight (pounds)	N	Suffolk Lambs[5] 10 Day Weight (pounds)	20 Day Weight (pounds)	60 Day Weight (pounds)
No Se	0	22	16.0 ± 0.8^a	22.0 ± 1.0^a	41.9 ± 1.8^a	9	17.7 ± 1.1^a	24.5 ± 1.5^a	48.2 ± 2.6^a	11	14.5 ± 1.1^a	19.9 ± 1.3^a	36.7 ± 2.1^c	15	14.6 ± 0.9^a	20.1 ± 1.3^a	39.4 ± 2.3^a	7	17.9 ± 1.4^a	24.9 ± 1.4^{ab}	45.6 ± 2.8^{ab}
Se-Yeast	0.3	14	15.1 ± 1.0^a	21.4 ± 1.2^a	41.7 ± 2.2^a	4	16.9 ± 1.6^a	22.6 ± 2.3^a	50.2 ± 4.1^a	9	14.0 ± 1.2^a	20.6 ± 1.4^a	38.4 ± 2.2^{bc}	7	15.4 ± 1.3^a	21.4 ± 1.7^a	42.7 ± 2.1^a	7	15.1 ± 1.6^a	21.8 ± 1.6^b	41.1 ± 3.1^b
	0.9	16	16.0 ± 0.9^a	23.2 ± 1.1^a	44.0 ± 2.1^a	5	16.3 ± 1.5^a	23.6 ± 2.1^a	44.2 ± 3.4^a	10	15.5 ± 1.2^a	22.6 ± 1.3^a	43.8 ± 2.2^a	12	14.3 ± 1.0^a	21.4 ± 1.3^a	41.1 ± 2.4^a	4	19.1 ± 1.9^a	26.8 ± 1.9^a	50.1 ± 3.7^a
	1.5	22	16.3 ± 0.8^a	22.4 ± 1.0^a	44.6 ± 1.7^a	5	17.8 ± 1.4^a	23.7 ± 2.0^a	50.3 ± 3.4^a	17	15.3 ± 0.9^a	21.5 ± 1.0^a	42.3 ± 1.6^{ab}	15	15.5 ± 0.9^a	20.7 ± 1.2^a	41.9 ± 2.0^a	7	17.0 ± 1.4^a	24.9 ± 1.5^{ab}	49.2 ± 2.8^a

[a,b,c,] Within a column, means without a common superscript differ ($P < 0.10$).
[1] Analysis comparing all lambs from ewes in all treatment groups.
[2] Analysis comparing lambs raised as singles from ewes in all treatment groups.
[3] Analysis comparing lambs raised as twins from ewes in all treatment groups.
[4] Analysis comparing lambs from Polypay ewes in all treatment groups.
[5] Analysis comparing lambs from Suffolk ewes in all treatment groups.

Table 7b. Year two lamb performance measures: 10-, 20-, and 60-day average daily gains (ADG) (least squared means ± SEM) of lambs from ewes dosed weekly with an organic Se source at varying dietary concentrations.

Se Source	Se dose, (mg/kg)	All Lambs[1]				Reared as Singles[2]				Reared as Twins[3]				Polypay Lambs[4]				Suffolk Lambs[5]			
		N	10 Day ADG (grams)	20 Day ADG (grams)	60 Day ADG (grams)	N	10 Day ADG (grams)	20 Day ADG (grams)	60 Day ADG (grams)	N	10 Day ADG (grams)	20 Day ADG (grams)	60 Day ADG (grams)	N	10 Day ADG (grams)	20 Day ADG (grams)	60 Day ADG (grams)	N	10 Day ADG (grams)	20 Day ADG (grams)	60 Day ADG (grams)
No Se	0	22	718 ± 36^a	499 ± 22^a	321 ± 13^b	9	761 ± 51^a	553 ± 35^a	366 ± 20	11	654 ± 50^a	452 ± 29^a	279 ± 16^b	15	663 ± 42^a	456 ± 28^a	266 ± 16^a	7	805 ± 64^{ab}	569 ± 32^{ab}	346 ± 20^{ab}
Se-Yeast	0.3	14	681 ± 44^a	489 ± 27^a	326 ± 16^{ab}	4	748 ± 76^a	509 ± 53^a	382 ± 32	9	636 ± 56^a	466 ± 31^a	289 ± 17^b	7	702 ± 58^a	488 ± 38^a	329 ± 22^a	7	651 ± 70^b	486 ± 34^b	318 ± 23^b
	0.9	16	712 ± 42^a	528 ± 25^a	344 ± 15^{ab}	5	717 ± 69^a	529 ± 47^a	340 ± 27	10	695 ± 53^a	513 ± 30^a	335 ± 17^a	12	652 ± 46^a	489 ± 30^a	316 ± 17^a	4	842 ± 85^a	600 ± 43^a	384 ± 27^a
	1.5	22	728 ± 34^a	513 ± 21^a	353 ± 12^a	5	799 ± 68^a	536 ± 47^a	382 ± 26	17	688 ± 45^a	427 ± 23^a	324 ± 12^a	15	708 ± 40^a	481 ± 26^a	331 ± 15^a	7	748 ± 64^{ab}	559 ± 32^{ab}	377 ± 20^a

[a,b,c] Within a column, means without a common superscript differ ($P < 0.10$).

[1] Analysis comparing all lambs from ewes in all treatment groups.

[2] Analysis comparing lambs raised as singles from ewes in all treatment groups.

[3] Analysis comparing lambs raised as twins from ewes in all treatment groups.

[4] Analysis comparing lambs from Polypay ewes in all treatment groups.

[5] Analysis comparing lambs from Suffolk ewes in all treatment groups.

Figure 7a. Year two lamb performance measures: 10-, 20-, and 60-day body weights (least squared means ± SEM) of lambs from Suffolk ewes dosed weekly with an organic Se source at varying dietary concentrations.

Figure 7b. Year two lamb performance measures: 60-day body weights (least squared means ± SEM) of lambs from Suffolk ewes dosed weekly 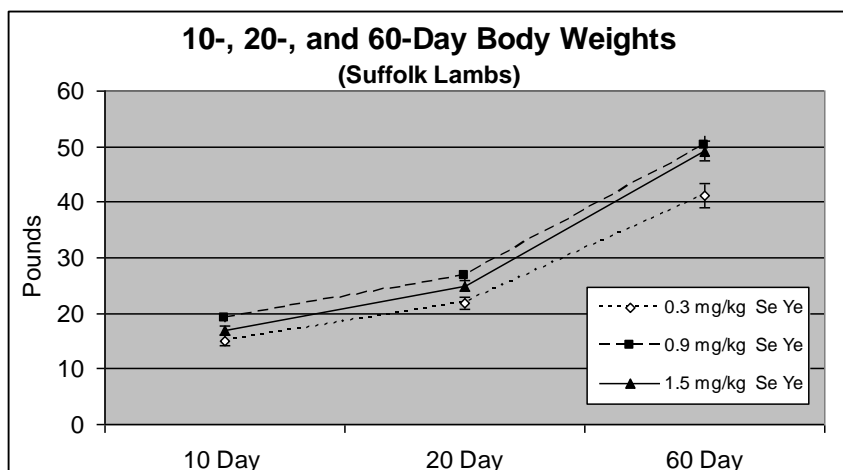with an organic Se source at varying dietary concentrations. [a,b]Bars without a common superscript differ ($P < 0.10$).

Figure 8a. Year two lamb performance measures: 10-, 20-, and 60-day body weights (least squared means ± SEM) of lambs reared as twins from ewes dosed weekly with an organic Se source at varying dietary concentrations.

Figure 8b. Year two lamb performance measures: 60-day body weights (least squared means ± SEM) of lambs reared as twins from ewes dosed weekly with an organic Se source at varying dietary concentrations. [a,b]Bars without a common superscript differ (*P* < 0.10).

Figure 9. Year two lamb performance measures: 60-day ADG (least squared means ± SEM) of lambs from ewes receiving no Se, or dosed weekly with an organic Se source at varying dietary concentrations. [a,b]Bars without a common superscript differ ($P < 0.10$).
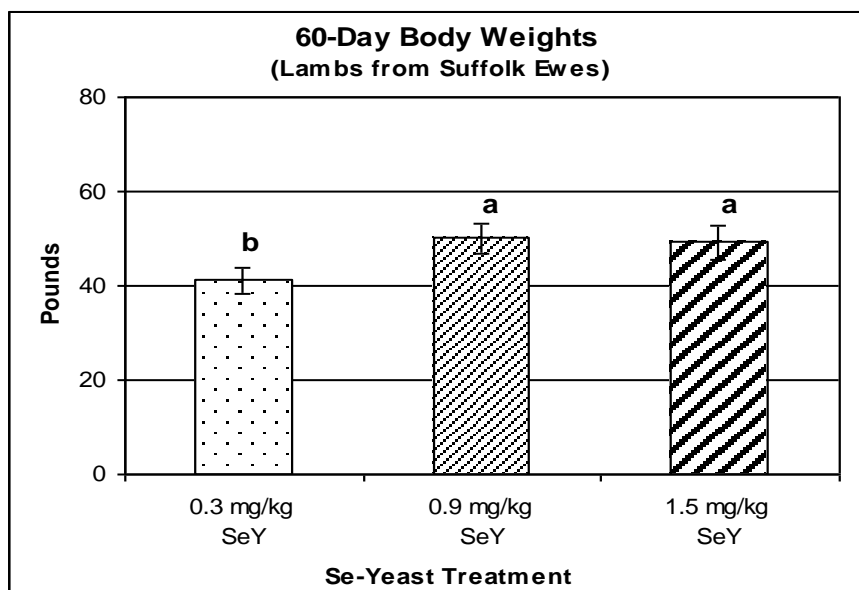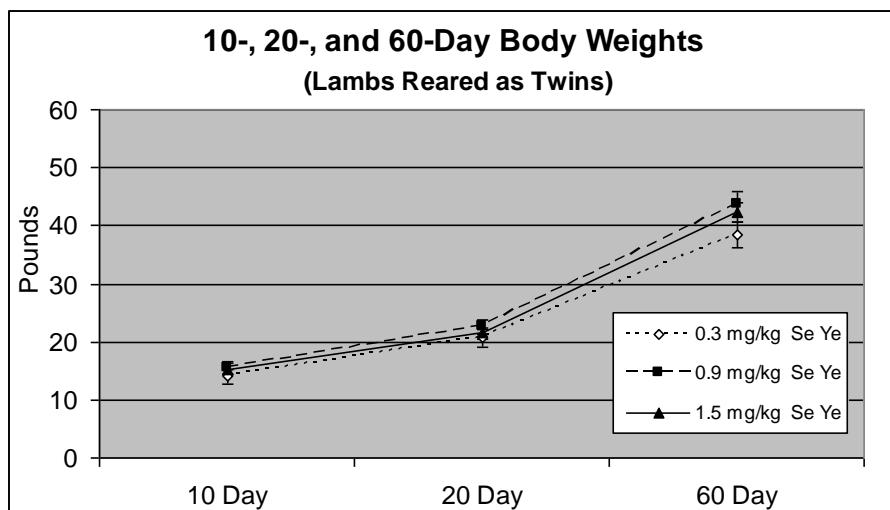

Figure 10. Year two lamb performance measures: 60-day ADG (least squared means ± SEM) of lambs reared as twins from ewes receiving no Se, or dosed weekly with an organic Se source at varying dietary concentrations. [a,b]Bars without a common superscript differ ($P < 0.10$).

Figure 11. Year two lamb performance measures: 60-day ADG (least squared means ± SEM) of lambs from Suffolk ewes receiving no Se, or dosed weekly with an organic Se source at varying dietary concentrations. [a,b]Bars without a common superscript differ ($P < 0.10$).
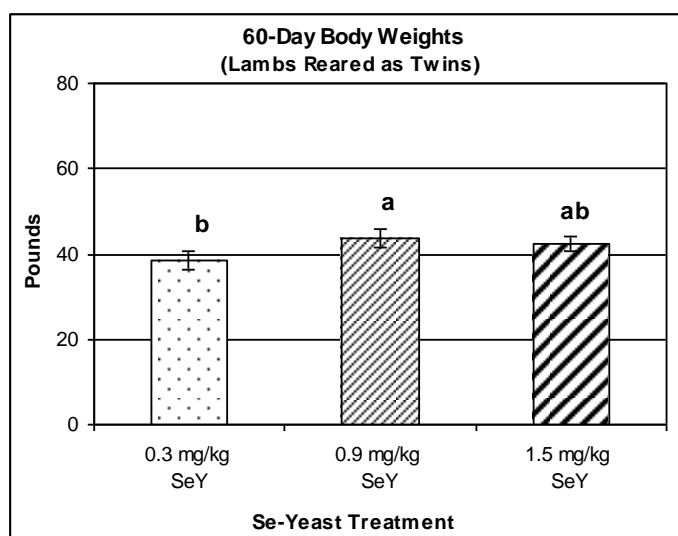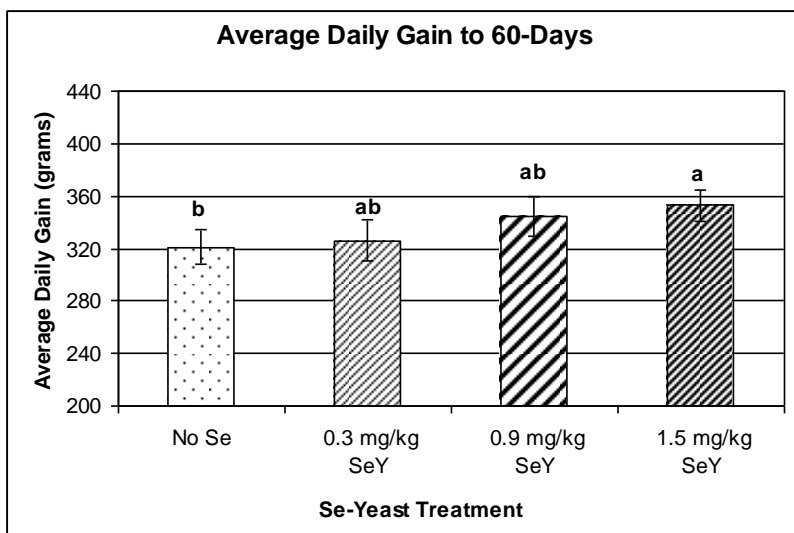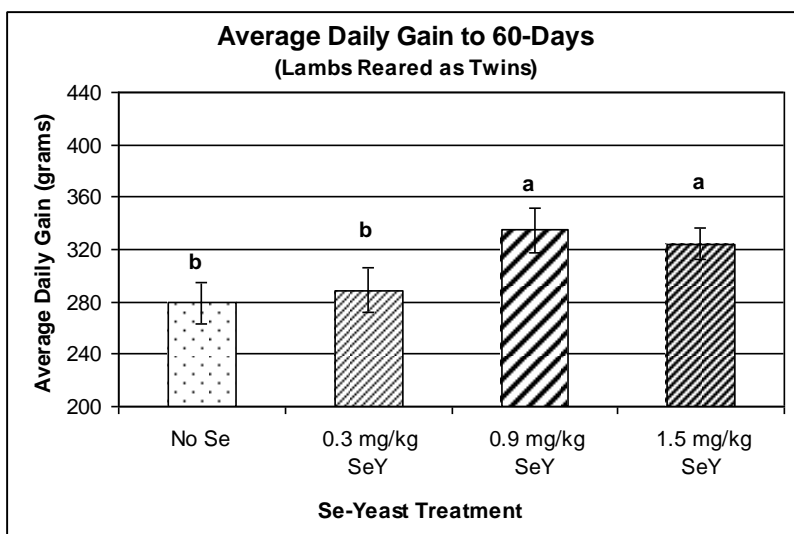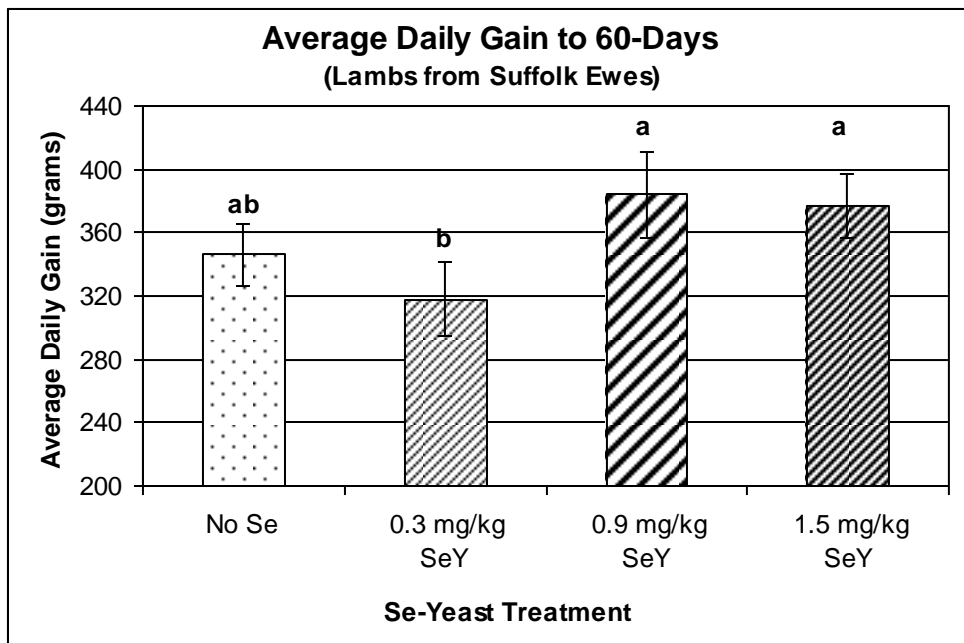
5.4.8 Year Two Lamb Performance Measures: Percentage of Lambs Surviving to 60 Days

The values for percentage of lambs surviving to 60 days are shown in **Table 8**.

Compared to lambs from ewes receiving no Se, lambs born to ewes receiving SeY at 1.5

mg/kg had a higher percentage of lambs surviving to 60 days ($P = 0.04$). However,

compared to lambs from ewes receiving 0.3 mg/kg SeY, lambs from ewes receiving SeY

at 1.5 mg/kg did not have a higher percentage of lambs surviving to 60 days ($P > 0.05$).

Table 8. Year two lamb performance measures: percentage of lambs surviving to 60 days in lambs from ewes dosed weekly with an organic SeY source at varying dietary concentrations. [a,b]Within a column, percentages without a common superscript differ ($P < 0.05$).

Percentage of Lambs Surviving to 60 Days				
Se Source	Se dose (mg/kg)	N = Birth	N = 60 Days	% Survival to 60 Days
No Se	0	36	23	64[b]
Se-Yeast	0.3	27	18	67[ab]
	0.9	30	20	67[ab]
	1.5	42	36	86[a]

5.5 DISCUSSION

5.5.1 Ewe Reproductive Performance Measures

In year one, Se supplementation, irrespective of source (i.e., selenium yeast,

sodium selenite, sodium selenate) and/or concentration (i.e., 0.3 mg/kg, 0.9 mg/kg, and

1.5 mg/kg), had no effect on lambing percentage, number of lambs born per ewe, or lamb

birth weights. In year two, Se supplementation with SeY, irrespective of

supplementation rate, had no effect on percentage of ewes exhibiting estrus, number of lambs born per ewe, or lamb birth weights.

Surprisingly, in the second year ewes receiving no Se had a higher percentage of ewes exhibiting estrus that lambed, and a higher percentage of ewes lambing compared with ewes receiving SeY at 0.3 mg/kg. It is possible that the SeY supplement might have negatively influenced early term fetal development. Although supranutritional Se supplementation with SeMet has not been reported to negatively affect ewe fertility by others (Swanson et al., 2008; Taylor et al., 2009; Munoz et al., 2008) no one has specifically investigated early fetal death in ewes given large oral dosages of SeY. It is possible that SeY provided as a concentrated once weekly oral drench is more toxic than a daily dietary Se supplement. Because trends were not consistent in both study years, further investigation is warranted to specifically investigate whether supranutritional SeY dosages negatively impact lambing percentages.

5.5.2 Newborn Lamb Vigor Scores

In year two, lamb vigor scores were assigned according to how rapidly after parturition the newborn lamb was able to stand and nurse. Lamb vigor appeared to be enhanced as a result of supranutritonal SeY supplementation. This was especially apparent in lambs from ewes receiving 1.5 mg/kg of SeY; their lambs had a quicker progression to stand and nurse than lambs from ewes receiving SeY at the 0.3 mg/kg and 0.9 mg/kg concentrations.

A faster progression to sucking relates to improved survival in lambs (Dwyer et al., 2001). Lamb survivability to 60 days was also higher in lambs from ewes receiving

SeY at 1.5 mg/kg in our study. This is in agreement with Munoz et al. (2008) who also observed greater lamb survival to 6 weeks of age ($P =0.09$) when ewes were fed SeY throughout pregnancy. However, our study is novel in that we measured lamb vigor and survivability as a result of ewes being supplemented with SeY at recommended (0.3 mg/kg) as well as supranutritional rates (0.9 mg/kg and 1.5 mg/kg) throughout gestation.

5.5.3   Lamb Growth Performance Measures

Because lambs did not receive any supplemental Se from birth to weaning in either study year, conclusions are based on the premise that differences in lamb Se status at birth between treatment groups would exist throughout the study period. Although whole-blood Se concentrations were not measured, the assumption made, based on a study by Juniper et al. (2008), is that Se clearance in lambs with whole-blood Se concentrations of 700 ng/mL would take approximately 300 days to decline to 200 ng/mL.

From our study, we conclude that providing supranutritional Se to pregnant ewes exerts a greater influence on lamb growth during periods approaching weaning rather than during neonatal growth periods. In year one, we observed the greatest influence of Se supplementation to ewes on lamb growth at 120 days in both organic and inorganic treatment groups for the 1.5 mg/kg concentration. This was also reflected in the 120 day ADG values in lambs from ewes receiving supranutritional Se supplementation. This trend of increased weight gain in lambs at 120 days was significant more consistently in lambs from Suffolk ewes ($P =0.07$), and in lambs reared as twins ($P =0.09$). In year two, we measured weight gains in lambs at 10-, 20-, and 60-days. Similar to year one,

differences in weight gain were more pronounced in older lambs (60 days) in year two. At day 60, there was significant difference in 60-day ADG when comparing lambs from the 1.5 mg/kg SeY group to the no-Se group ($P = 0.07$). Again, this supranutritional Se influence on 60-day ADG was most pronounced in lambs from Suffolk ewes ($P = 0.06$), and in lambs reared as twins ($P = 0.03$). We believe that differences in growth were noticeable in lambs at earlier ages in year two (60 days) compared to year one (120 days) because of the declining Se status of ewes in the no-Se group in the second consecutive year. Consequently, lambs from ewes in the no-Se group would have been born with a lower Se status in year two and, therefore, would have begun to exhibit Se-responsive growth depression at an earlier time point.

Because the greatest requirement for Se is for growth (NRC, 2007), this explains why differences were detected based on the two sheep breeds utilized in our study. It is possible that the effects of Se were more noticeable in lambs from Suffolk ewes because of the larger frame size and more rapid growth characteristics of the Suffolk breed. The differences in growth detected in lambs reared as twins compared to lambs reared as singles might be explained by Se dilution in the milk when consumed by two lambs vs. one lamb. Munoz et al. (2008) found that Se requirements of ewes rearing multiple lambs were greater than those for ewes rearing single lambs because Se was diluted with multiple lambs. However, based on milk-Se concentrations (discussed in Chapter 3), it is unlikely that Se concentrations in milk beyond 30 days provide adequate Se to growing lambs.

It is possible that the enhanced weight gains observed in both years in lambs from ewes receiving SeY was a function of enhanced feed efficiency. Zhan et al. (2010) found that maternal SeMet intake had a more positive effect on the degradation and absorption of nutrients in piglets. Compared with sodium selenite, maternal SeMet intake increased protease, amylase, and lipase activities in pancreatic tissues of offspring (Zhan et al., 2010). Neville et al. (2010) observed increased vascularity in the jejunum of lambs born from ewes receiving supranutrtitional concentrations of SeY compared to ewes receiving SeY at recommended levels, which might influence nutrient absorption in the small intestine.

In light of ours and previous studies (see Chapter 3), we can assume that as time progressed and Se concentrations were depleted in ewes receiving no Se supplement, a difference in lamb growth as a result of Se status was observed. Although the SeMet content of our SeY supplement was not analyzed, numerous studies have shown that SeMet is the primary source of Se (63%) found in most Se yeast supplements (Juniper et al. 2008). Selenomethionine is retained two times longer than Se administered as sodium selenite (Rayman et al., 2008). Therefore, in our study we can assume that lambs born from ewes receiving inorganic Se sources were Se-depleted more rapidly than lambs born from ewes receiving organic SeY. This might explain the greater growth rates in lambs from ewes supplemented with organic SeY compared to lambs from ewes receiving inorganic sodium selenite.

Tiwary et al. (2006) suggested that tissue storage of Se given as SeMet can be used advantageously in animals that require a sustained Se source, because Se is released

from structural selenoproteins during the process of protein turnover. It is possible that over time, the Se stored as SeCys in lambs from ewes given sodium selenite became depleted, whereas lambs from ewes given SeY had sufficient Se stored in muscle for optimal growth and biological function as time progressed. In our study, it is possible that differences in the amount of Se contained in muscle (i.e., SeMet or SeCys) contributed to Se clearance and the limiting effect of Se on growth. We can only speculate that lambs from ewes receiving inorganic sodium selenite had different amounts of Se as SeMet and SeCys in skeletal muscle compared with lambs from ewes given SeY. The rate of Se clearance in sheep varies according to the tissue involved and the Se species present in the tissue (Juniper et al., 2008). Consequently, we conclude that the merits of supranutritional Se supplementation in terms of lamb growth is in the capacity to store and supply Se during critical periods of lamb growth. This was best achieved in our study by providing Se as SeY.

Discrepancies among studies looking at lamb growth performance in response to inclusion of Se in the diet exist. Possible causes for the Se discrepancies include the chemical form of Se administered, the supplementation rate, the Se status of treatment groups, the age of lambs studied, and the route of Se administration. Similar to our study, previous studies where lamb growth was measured as a result of Se supplementation to pregnant ewes have consistently shown enhanced growth in lambs (Abd El-Ghany et al., 2008; Gabryszuk and Klewiec, 2002; Munoz et al., 2008). Abd El-Ghany et al. (2008) and Gabryszuk and Klewiec (2002) showed that lamb growth as a result of Se supplementation to the pregnant ewe was most noticeable in the first two weeks of age,

although other studies have shown that enhanced growth from Se supplementation can be detected in lambs up to one year of age (Munoz et al., 2008; Kumar et al., 2009).

5.6   REFERENCES

See Chapter 7.

CHAPTER 6.   CONCLUDING THOUGHTS

Selenium's involvement in ruminant nutrition is complex. It is therefore challenging to measure all the effects it may exert when different Se sources are used and supplemented at supranutritional concentrations. Clearly we learned valuable information from the studies discussed previously both for producer application and future research directives.

We now have a better understanding the dynamics of Se transfer. We understand that placental and mammary transfer of Se is achieved with an increasing rate of supplementation. Furthermore we understand that organic sources such as Se yeast (SeY) are more readily transferred than inorganic selenium sources such as sodium selenite which are limited in their transfer capabilities. Furthermore by measuring the transfer of whole-blood and serum Se concentrations of the newborn lamb we better understand that the Se found in the serum fraction is prioritized and more regulated than the Se found in the whole-blood fraction. We also learned that Se drenching of ewes on weekly basis is an effective method of achieving elevated Se status of the new-born lamb. Consequently sheep producers might better improve their current strategies of how to provide Se during the most critical production periods most efficiently saving valuable time and economic resources.

We also observed that Se deficiency in ewes and lambs was most apparent after the second consecutive year of not receiving Se. In ewe production measures we observed that lambing percentages were significantly lower the second year in groups not

receiving Se. These deficiency impairments were also reflected in lamb vigor scores and lamb survival rates to 60 days. Our study design in measuring lamb growth attempted to mimic a production scenario where newborn lambs at birth had to rely solely on Se acquired from their dams. Thus attempting to better identify during what periods of neonatal growth are most influenced by Se status. In both years we observed that lamb growth performance was most affected in the later periods of lamb growth when Se reserves from birth became limited.

Finally our research findings on whether passive immunity was influenced by Se source or maternal supplementation rate yielded less conclusive results. Had we controlled certain experimental variables such as measuring the amount of colostrum the lamb consumed we could have accurately measured IgG absorption and drawn more definitive conclusions. However by measuring the IgG concentrations in the ewe colostrum and the lamb serum at 48 hours we observed small numerical increases. Specifically that sodium selenite tends to increase IgG concentrations in lamb serum up to a specific 0.3 to 0.9 mg/kg threshold. In contrast with SeY we observed consistent increases in both colostral and lamb serum IgG concentrations as the supplementation rate increased. Thus we can speculate that the Se source does influence aspects of passively transferred immunity, although future research is warranted.

Involvement of other essential nutrients all contribute to the productivity of an animal. Selenium plays a multi-faceted role on biological function, which may or may not have been entirely detected by the measures in our study. For example, more than 30 seleno-proteins have been identified in various mammalian tissues, although only 20 have

known biological functions. These seleno-proteins are categorized into three groups according to their involvement in cellular functions: 1) the glutathione peroxidases (GSHPx) responsible for reducing hydroperoxides in cells, 2) the thioredoxin reductases involved in cell growth and apoptosis, and 3) the iodothyronine deiodinases involved in thyroid hormone metabolism. Our study did not measure the specific seleno-proteins or their interaction with Se transfer, passive immunity or ewe and lamb production measures. Therefore future research may be best directed at understanding how the involvement of these various seleno-proteins are influenced by the different Se sources (e.g., sodium selenite, sodium selenate and selenized yeast) and rate of supplementation.

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