

January 2015

# Effects of Organic and Inorganic Selenium Supplementation during Late Gestation and Early Lactation on Beef Cow-Calf Performance

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**PURDUE UNIVERSITY  
GRADUATE SCHOOL  
Thesis/Dissertation Acceptance**

This is to certify that the thesis/dissertation prepared

By Christopher R. Muegge

Entitled

EFFECTS OF ORGANIC AND INORGANIC SELENIUM SUPPLEMENTATION DURING LATE GESTATION AND EARLY LACTATION ON BEEF COW-CALF PERFORMANCE

For the degree of Master of Science

Is approved by the final examining committee:

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Ron Lemenager

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10/12/2015

Date

EFFECTS OF ORGANIC AND INORGANIC SELENIUM SUPPLEMENTATION DURING LATE  
GESTATION AND EARLY LACTATION ON BEEF COW-CALF PERFORMANCE

A Thesis

Submitted to the Faculty

of

Purdue University

by

Christophert Robert Muegge

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

December 2015

Purdue University

West Lafayette, Indiana

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## ABSTRACT

Muegge, Chris R., M.S., Purdue University, December 2015. Effects of Organic and Inorganic Selenium Supplementation during Late Gestation and Early Lactation on Beef Cow-Calf Performance. Major Professor: Jon Schoonmaker

Angus x Simmental cows (n = 48, BW = 594 kg, BCS = 5.26, Age = 2.7), pregnant with male fetuses, were used to determine the effect selenium (Se) source during the last 80 d of gestation and first 108 d of lactation on progeny feedlot performance. At 203 d in gestation, cows were blocked by body weight, age, breed composition, and calf sire, and allotted to 1 of 3 treatments: no Se, inorganic Se, or organic Se. Maternal diets were formulated to contain 10.4% CP and 0.90 Mcal/kg NEg during gestation and 12.1% CP and 1.01 Mcal/kg NEg during lactation. Basal diets contained 0.07 and 0.11 mg/kg Se for gestation and lactation diets; respectively. Diets were fed daily as a total mixed ration and Se was provided in a top-dress containing 0, 0.30 mg/kg Se as sodium selenite, or 0.30 mg/kg Se as Sel-Plex<sup>®</sup>. Treatment diets were fed through 108 d post-partum (DPP). At 68 DPP milk production was calculated using the weigh-suckle-weigh procedure and a milk sample was collected was at 62 DPP to determine composition. At 108 DPP cow-calf pairs were commingled until weaning at 216 DPP. At 28 d post-weaning, steers (n = 47, BW = 301 kg) were placed in individual pens and fed a diet formulated to provide 13.9% CP, 1.24 Mcal/kg NEg, and 0.10 mg/kg Se. The diet was

delivered as total mixed ration once daily. Cow weight and BCS and calf birth weight did not differ at the beginning of the trial ( $P \geq 0.55$ ). Cow BW and BCS ( $P \geq 0.85$ ) did not differ between treatments at any time point during the study. Milk production, milk fat, and total solids ( $P \geq 0.38$ ) did not differ among treatments. Milk protein tended to increase in cows fed inorganic Se compared to cows fed organic Se ( $P = 0.07$ ) and milk lactose tended to be greatest in cows fed organic Se ( $P = 0.10$ ). Conception to AI and overall pregnancy rates did not differ between the diets ( $P \geq 0.39$ ). Calf weights and ADG did not differ for the 105 d experimental period ( $P \geq 0.77$ ) or for the entire pre-weaning period ( $P \geq 0.33$ ). Plasma Se concentration did not differ between treatments for the cows ( $P \geq 0.37$ ). Liver Se concentration in cows fed inorganic and organic Se treatment were significantly greater than that of cows from the control treatment ( $P < 0.01$ ). There were no treatment effects on calf plasma Se concentration ( $P \geq 0.90$ ). Muscles biopsies taken at 105 DPP also were not different ( $P = 0.45$ ). Steers from cows supplemented with organic Se diet entered the feedlot heavier ( $P = 0.02$ ) and tended to be heavier on d 87 ( $P = 0.08$ ) compared to steers from cows supplemented with inorganic Se. There was no difference in ADG among treatments ( $P \geq 0.76$ ), but steers from organic Se cows tended to spend fewer days on feed compared to steers from inorganic Se cows ( $P = 0.09$ ). Steers from organic Se cows had a greater overall DMI compared to steers from inorganic Se cows ( $P = 0.04$ ), but there was no difference in overall gain:feed ratio ( $P = 0.82$ ). Dressing percentage was greater for steers from cows fed no Se compared with steers from cows fed either inorganic or organic Se ( $P = 0.03$ ). Maternal Se source had no effect on hot carcass weight, back fat, %KPH, L. dorsi area, yield grade, marbling

score, or quality grade distribution ( $P \geq .17$ ) of progeny. In conclusion, dietary Se source did not affect cow performance, milk production, or reproductive ability. Organic Se decreased milk protein and increased milk lactose, but did not alter pre-weaning performance of the progeny. Maternal supplementation with organic Se appears to have a long-term benefit on intake of steer progeny and may result in improvements in growth that decreases days in the feedlot.

## CHAPTER 1 LITERATURE REVIEW

### 1.1 Role of Selenium

Selenium (Se) has traditionally been known for its role in antioxidant defense. It was first recognized for its immune function in 1959, when it was found to be incorporated into a protein in leukocytes of dogs. In 1973, scientists discovered the role Se plays as part of the enzyme glutathione peroxidase (GPx), which is a selenoprotein that detoxifies harmful organic hydrogen peroxides (McKenzie et al., 1998). There have been over 26 different selenoproteins identified in Eukaryotes, although the roles of all 26 are not fully known. Of those selenoproteins that are known, all play a role in oxidoreductase intervening enzymes implicated in multiple metabolic pathways (Rederstorff et al., 2006). Selenium is incorporated into selenoproteins as selenocysteine when it is formed in the liver. After Se is absorbed from the diet, it is associated with plasma proteins in the blood supply and transferred to the liver where it replaces S to form selenocysteine (Symonds et al, 1981). However, Se is thought to chelate with at least 20 different amino acids.

As animals are exposed to different stresses, toxins, and injuries throughout their life, there is a buildup of reactive oxygen species (ROS), such as hydrogen peroxide. This increase in ROS production leads to oxidative damage which can negatively affect

cellular functionality by harming cellular lipids, proteins and DNA (Miller et al., 1993). Glutathione Peroxidase is key to the prevention of oxidation reactions and the buildup of free radicals such as peroxides. Glutathione peroxidase works by reducing the hydrogen peroxide into water, thus preventing oxidative damage (Finch and Turner., 1996). Selenoprotein W is thought to play a role in muscle development and maintenance and white muscle disease. Muscle from Se-supplemented lambs contains selenoprotein W while lambs known to be Se deficient, and displaying signs of white muscle disease showed only trace amounts of selenoprotein W (Black et al., 1978). Another selenoprotein that seems to be involved in muscle development includes selenoprotein N. Although, the exact role is not clear, selenoprotein N is believed to be involved in the development of muscular dystrophy (Rederstorff et al., 2006). Multiple selenoproteins are involved in thyroid metabolism. Selenium and vitamin E injections to newborn Holstein calves catalyzed deiodinase activity, improving the conversion of the inactive  $T_4$  to the active  $T_3$ . The thyroid plays a role in regulation of hepatic enzyme expression and neutrophil function, thus Se helps to maintain proper hormone balance in the thyroid and promote proper development and growth (Rowntree and Hill, 2004). A decrease in the ratio of the  $T_3/T_4$  ratio in blood is a significant sign of aging. However, Se supplementation has been reported to reverse the decrease in  $T_3/T_4$  (Olivieri et al., 1995). Approximately 60% of selenium available in plasma is in the form of selenoprotein P while an additional 30% of plasma Se is made up of extracellular GPx (Brown and Arthur, 2001). Selenoprotein P and plasma GPx are the only known selenoproteins found in plasma (Burk R.F., 1994). Selenoprotein P is found in many

tissues of the body along with several membranes linking it to the transportation of Se and antioxidant defense (Burk, 1994).

Vitamin E's role in antioxidant properties focuses on the protection of polyunsaturated fatty acids in cell membranes from lipid peroxidation by quenching and scavenging reactive oxygen species and thereby preventing the formation of hydroperoxides (Mascio and Murphy, 1991). Therefore, vitamin E works in concert with Se to help maintain low cellular and tissue concentrations of ROS and increased cell function (McDowell et al., 1996). The relationship between vitamin E and GPx activity, however, has been much more controversial. Gatellier et al. (2004) reported a significant negative correlation between vitamin E concentrations and GPx activity, even though it is known that vitamin E has the ability to quench free radicals capable of propagating lipid peroxidation and improve neutrophil health (Grady et al., 2001). In addition, supplementation of vitamin E in the diet decreases the susceptibility of muscle and myoglobin to oxidation (Mitsumoto et al., 1998). Yao et al. (2015) also reported a synergistic effect for the combination of Se and Vitamin E supplementation in rats. Increases in antioxidant activity through elevated concentrations of superoxide dismutase, GPx and glutathione production were reported when the combination of Vitamin E and Se was supplemented compared to either supplement alone (Yao et al., 2015). The combination of Se-dependent GPx and proper vitamin E concentrations improve the antioxidant defense capability of beef animals.

Glutathione peroxidase production is dependent on selenocysteine availability, and therefore the selenium concentration in the diet has a direct effect on GPx activity (Walsh et al., 1993). Proper Se concentrations improve GPx activity which can then function with another antioxidant, vitamin E, to control peroxidation (Ilad et al., 2001). A lack of Se availability will lead to an increased incidence of oxidative damage. In turn, this will lead to cell membrane breakdown, decreased fertility, and poor growth. Furthermore, deficiencies in Se availability will often result in decreased activity of other selenoenzymes, however some selenoenzymes will actually increase in activity when Se seems to be deficient. In this case, these selenoenzymes are thought to be critical to the survival of the animal (Wichtel, 1998).

### 1.2 Dietary Selenium

The Beef Nutrition Research Council (NRC; 1996) states that cattle require at least 1.0 mg/d of Se to ensure normal function. However, supplementation is limited to no more 3.0 mg/d by the Food and Drug Administration, because of the variability in soil Se concentrations across the U.S. which can cause either a deficiency or toxicity. Deficiency symptoms include nutritional myodegeneration, decreased performance, early-embryonic death, decreased fertility, and weak calves at calving (Corah, 1996). Livestock can also suffer from subclinical symptoms when consuming diets low in Se. In these cases, performance may only be slightly affected, however, when exposed to a stressor, more serious problems can present themselves.

Substantial research has looked at the benefits of providing Se to livestock. This work consistently suggests that Se plays a vital role in the health of the animal, allowing for proper health, fertility, and growth. However, appropriate Se concentrations in ruminants have been heavily contested with little consensus. In general, it is agreed that Se concentrations below 50 ng/ml of whole blood will result in Se deficiency symptoms (Hintze et al., 2001). However, subclinical symptoms of Se deficiency have been observed in animals with whole blood Se content ranging from 60 – 100 ng/ml (Corah, 1996). If an individual animal suffers from subclinical symptoms, any type of physical or nutritional stressor can trigger much more severe symptoms. This is especially important as these animals go into the weaning phase where both physical and nutritional stressors will be induced. It has also been concluded that adequate Se concentrations are reached at 100 ng/ml of whole blood (Koller and Exon, 1986). However, more recent research has suggested that whole blood concentrations as high as 200 ng/ml may be required for Se adequacy (Kincaid, 1999). This variation in results has led to little consensus for proper Se supplementation and has led some to believe that appropriate Se status is dependent on the individual animal and its environment. Discrepancies in appropriate Se status in the literature has also led to more questions about the bioavailability and retention capacity of different forms of Se products. Recent work has suggested that ruminants are able to withstand higher concentrations of supplementation depending on supplemental form of Se (Lawler et al., 2004; Hall et al., 2012).

### 1.2.1 Selenium Toxicity

Selenium toxicity can be seen when Se is fed at concentrations above 5.0 mg/d for extended periods of time and symptoms include “blind staggers,” anemia, roughened hair coat, stiffness, and hoof deformities (Koller and Exon, 1986). However, recent reports have shown that Se fed as high as 10.0 mg/d through Se enriched wheat (organic form of selenomethionine), showed no toxicity effects (Hintze et al., 2002). Mechanisms behind Se toxicity are not fully understood, however, excess concentrations of inorganic Se sources react with thiols to form seleno-trisulphides, which can form oxygen free radicals. Excess Se can also have an inhibitory effect of thiol production (Mézes and Balogh, 2009).

Schrauzer (2003) suggests that feeding organic Se sources allows beef animals to tolerate higher concentrations of Se without the negative side effects of Se toxicity. This is due to selenomethionine being stored in the body as methionine. Like most amino acids, the body is capable of storing large quantities of methionine. When dietary Se exceed Se needs, it is stored in proteins in place of methionine therefore decreasing some of the Se toxicity risks (Schrauzer, 2003). Researchers at North Dakota State University fed diets steers that provided over nine times the FDA regulated maximum concentrations through high-Se forages. These forages were grown in areas of high soil Se concentration and provided supranutritional concentrations of organically bound Se (selenomethionine) in the plant. Animals did not experience negative side effects, while increasing their own tissue Se concentrations (Lawler et al., 2004).

### 1.2.2 Selenium Deficiency

One of the classic results of a Se deficiency is white muscle disease (WMD). This is caused by the degeneration of striated muscle and is common in young, fast-growing ruminants in areas of low Se availability (McDowell et al., 1996). White muscle disease results in muscle weakness and the inability of the animal to stand, often leading to death without proper therapeutic Se supplementation. If an animal becomes deficient in Se and/or vitamin E, ROS accumulate and cause cell membrane damage and defective ion exchange and nutrient transport. Defective ion exchange then causes a buildup of calcium in the cell leading to mitochondrial damage and the inability of the cell to achieve homeostasis (Abutarbush and Radostits, 2003). In turn, cell necrosis and cell death will occur (Abutarbush and Radostits, 2003; Rederstorff et al., 2006). If a cow is severely Se deficient, her offspring is likely to die *in utero*. If the offspring is not stillborn, then death as a result of heart failure from severe damage to the heart muscle and the inability to continue circulate blood flow occur very early in life (McDowell et al., 1996). Subclinical Se deficiency can also be a problem, where clinical signs may only become manifested during stressful periods in an animal's life. Activities such as handling, shipping, or diet changes can be precipitating factors. Mature ruminants can become unthrifty and suffer from nutritional myopathy and anemia when diets are consistently deficient in Se (Underwood 1981; Morris et al. 1984). Selenium has also been linked to proper thyroid metabolism. Becket et al. (1993) demonstrated that the selenoprotein family of reductases are responsible for the reduction of iodide compounds. A lack of Se

can result in a buildup of iodide and a biological inactive form of triiodothyronine. This buildup will result in hyperthyroidism and hormone imbalances in the thyroid which can lead to decreased thyroid function and poor growth and development (Beckett et al., 1993; Wichtel et al., 1996).

### 1.3 Fertility and Reproduction

Selenium availability has been associated with fertility and reproductive performance. Work has shown that Se-deficient cows have an increased post-partum interval compared to those supplemented with Se and Vitamin E (Aréchiga et al., 1994), which could decrease re-breeding performance and result in increased culling rates for the herd. Reasons for decreased reproductive performance due to Se deficiency are variable and could be a result of impaired hormone production, cystic ovaries, or retained placentas. Glutathione peroxidase plays a role in the arachidonic acid cascade in the production of prostaglandins. Deficiencies in Se concentrations would result in decreased GPx availability which could then disrupt the synthesis of prostoglandin (Wichtel, 1998), and thus limit follicular development and conception rates. Therefore, increased Se availability during gestation and lactation could lead to increased selenoprotein activity and improved antioxidant defense, which could then increase the health and performance of both the cow and the calf.

Increased rates of retained placentas have been associated with cows suffering from Se deficiency. Retained placentas occur when the fetal placenta fails to separate from the maternal placenta, often resulting in uterine infections (Wetherill, 1965). A

number of physiological, nutritional, and pathological effects can take place as a result of retained placenta, which can cause a further decrease in conception rates along with increased herd culling rates (Wetherill, 1965). Early work with Se supplementation and incidence of retained placenta, suggested rates as high as 50% in areas of low soil Se concentrations (Trinder et al., 1969). At this time, the normal occurrence of retained placentas in North America is thought to be 10% (Wetherill, 1965). The occurrence of retained placentas in these areas has been decreased through proper Se and vitamin E injections (Julien et al., 1976). Increased Se supplementation to dairy cows decreased the rate of retained placentas by 38% as compared to cows deficient in Se (Julien et al., 1976). The direct relationship between retained placenta and Se concentrations is not completely known due to the variety of issues that can lead to retaining placental tissue. However, oxidative stress and decreased hormone activity, specifically steroid synthesis from cholesterol, are both correlated with the incidence of retained placentas and decreased concentrations of both vitamin E and Se (Miller et al., 1993). Buck et al (1981) reported the Se is preferentially incorporated into the placentome, ovary, pituitary, and adrenal glands suggesting a specific need for this element in these female reproductive tissues. Therefore, a lack of Se could result in reproductive dysfunctions such as retained placenta (Buck, et al., 1981). Laven and Peters (1996) reported on the role of immune function with retained placentas. Leucocytes in the placentome aid in separation of placental tissues from the uterus and placentomes of cows with no leucocytes failed to separate from placental tissue (Laven and Peters, 1996). A decrease of chemotactic activity in leucocytes has been shown to result from decreased Se availability. A low

concentration of chemotactic activity was also found in cows retaining their placenta and an absence of leucocytes in the cotyledons was associated with an increased incidence of retained placentas (Laven and Peters, 1996).

Research has also demonstrated a decrease in the incidence of cystic ovaries due to Se supplementation. Animals injected with 0.1 mg/kg of Se 21 days prior to calving recorded a 31 % decrease in the incidence of cystic ovaries compared to the control group while animals supplemented with both vitamin E and Se experienced no cystic ovaries (Harrison et al., 1984). The requirement for Se is also increased during spermatogenesis because of its role in the production of GPx4. Low concentrations of Se at the onset of spermatogenesis may impair the sperm's mitochondrial capsule leading to decreased mobility and possible infertility (Brown and Arthur, 2001).

#### 1.4 Immune Function

Immune function is also correlated with the availability of Se. Selenium impacts cellular immunity via selenoprotein involvement in antioxidant defense, thyroid hormone metabolism, and the regulation of redox-active proteins (Mckenzie et al., 1957). Selenium boosts cellular immunity by enhancing T-cell responses, preventing oxidative immune cell damage, and altering platelet aggregation (Arthur et al., 2003). *In vitro* Se supplementation of lymphocytes collected from Se deficient lambs has been shown to be very effective at raising a lymphocyte response (Turner and Finch, 1990). This included a decrease in creatine kinase concentrations in lambs subjected to muscle myopathy when supplemented with Se and vitamin E. Furthermore, Turner and Finch

(1990) found that *in vivo* Se supplementation of lambs and calves improved lymphocyte response when these animals were under the age of 6 weeks, however, as animals increase in age this response tends to decrease (Turner and Finch, 1990). Whanger et al. (1978) attributed this decrease in lymphocyte response as animal's age to rumen development. As the rumen develops, ruminal microorganisms capable of metabolizing inorganic Se compounds increase in number, which limits the possible benefits that Se supplementation may have on immune function.

Selenium concentration by itself in the body does not reveal much about its functionality, whereas a measure of GPx activity provides more information. Glutathione peroxidase decreases oxidative stress and inflammation in the cell membrane (Malbe et al., 1995) by converting free radicals into more stable compounds like water. Therefore, GPx activity is often monitored and linked to improved immune function when examining Se status. For example, feeding cattle diets depleted in Se and Vitamin E over a 50 week period resulted in substantially decreased erythrocyte GPx activity, as well as an increase in subclinical myopathy compared to cattle supplemented with Se (Walsh et al., 1993). An increase in GPx activity was noted after only 60 days for yearling steers fed adequate Se concentrations in their feed compared to steers fed diets deficient Se (Arthington, 2008). Although GPx measurements can provide some indication of Se status, several other factors also determine GPx activity, limiting its utility as a sole measure of Se availability. Steers consuming adequate dietary Se also had greater liver and plasma Se concentrations compared to Se deficient steers

(Arthington, 2008). These results were similar to work performed in an Arkansas cattle herd where cows, offered Se as Se-yeast or sodium selenite in their free choice mineral, had elevated GPx activity compared to cows not supplemented (Gunter et al., 2003). In addition, Gunter et al. (2003) observed that calves from cows supplemented with Se-yeast had improved GPx activity as compared calves from sodium selenite supplemented cows (Gunter et al., 2003). Similarly, after 90 days of Se supplementation to goats, an increase in GPx activity was noted as compared to goats not supplemented with Se (Pavlata et al., 2011).

### 1.5 Selenium Sources

There is large variability in soil Se concentrations throughout the U.S. The primary source of Se for grazing animals is through forages grown on areas of differing soil Se concentration. Without supplementation, Se concentrations in grazing ruminants are dependent on the Se availability in the soil in their geographic location. Beef animals raised in areas of higher soil Se concentrations tend to have higher tissue concentrations of Se while those animals raised in areas of lower soil Se concentrations had consistently lower tissue Se concentrations (Hintze et al., 2001). Harvested forages and grains can be a good source of Se depending on soil Se concentrations. However, long storage time, moisture, sunlight, and temperature variation can decrease Se concentrations (Wichtel, 1998). Regions where soil Se concentrations have fallen below 0.5 mg/kg of dry weight are considered deficient and proper supplementation is crucial to the health of animals consuming forages from these areas (Wichtel, 1998). In general,

areas of the western United States tend to have elevated soil Se concentration that can meet or exceed animal requirements, while the Midwest and southeast generally have low concentrations of soil Se (Hintze et al., 2001). One report states that in areas of the southeast U.S., only 62.7% of cow herds are adequate in Se while 89.7% and 94.6% of herds in the western and central US, respectively, are adequate in Se (Dargatz and Ross, 2014).

Slow growing plants with deep roots tend to have the greatest uptake of soil Se. However, during times of rapid pasture growth, such as early spring, Se uptake will be limited (Grace and Wilson, 2002). Land that is considered to be sufficient in Se can be negatively affected by soil management. Selenium is not a requirement for plant growth, therefore attempts to improve soil fertility, through aeration and fertilization can limit the uptake of Se by naturally growing plants. Increasing soil aeration through tilling or cover crop production can decrease Se (Wichtel, 1998) because exposure to air and moisture results in the loss of stored Se. The application of sulfur in fertilizer can decrease Se availability (Wichtel, 1998) because sulfur can bind to Se making it unavailable for animal absorption. High sulfur concentrations in diets, which can be common when feeding many corn by-products, may limit the ability of the animal to absorb Se. Selenium and sulfur share very similar structures, resulting in decreased absorption of Se when sulfur is in competition. When sulfur concentrations become too high (above 0.30% of the diet) the ability of the body to retain Se is diminished.

### 1.5.1 Supplemental Forms

The traditional form of Se supplementation in livestock has been with the inorganic salt, sodium selenite. The utilization of inorganic minerals is dependent on the ability of the animal to ingest and then convert the salt to an active, organically bound form (Spears, 1996). Research has shown that animals have a limited ability to absorb and retain minerals that are supplemented as inorganic salts. This becomes particularly apparent in ruminants because of the strong reducing nature of the rumen, which reduces the Se compounds to insoluble selenides, which are excreted in urine and feces (Wright and Bell, 1966; Spears and Spears, 2003). Selenium is most commonly excreted in the feces but can also be excreted through the lungs and kidneys (Wichtel, 1998). Reduction of selenite can also result in an accumulation of insoluble compounds which can increase the risk of toxicity symptoms and decreased performance. Buildup of selenides in the system can react with thiols resulting in the production of superoxides and inhibiting some protein receptors (Wichtel, 1998). A more absorbable source may allow higher concentrations of Se supplementation without the risk of toxicity effects.

Due to concerns over the decreased bioavailability of inorganic salt supplements, many new organic mineral sources (Selenized yeast, zinc methionine, copper lysine) have been developed and marketed on the basis of increased absorption and retention capacity. Organic mineral sources are often referred to as chelated products because they are a complex between an organic ligand and a metal. The ligand-metal complexes are stable and are able to stay intact within the digestive tract allowing for increased

absorption in the small intestine. Commercially available organic minerals are in the same form in which they are found in the body and also found naturally in forages and grains, which is most commonly selenomethionine and selenocysteine. Organic Se is currently being commercially produced as a selenized yeast product by growing yeast in a Se-enriched environment. The yeast is able to incorporate Se into its cells by converting inorganic Se into selenomethionine. Increased absorption and retention of Se is achieved in organic products because of the nonspecific incorporation of selenomethionine into proteins in place of methionine (Spears and Spears, 2003; Spears, 1996). In selenized yeast, sulfur is replaced by Se in methionine resulting in increased absorption in both the muscle and milk of ruminants (Pehrson et al., 1999; Juniper et al., 2008). Since selenomethionine is recognized by the body as an amino acid, it is scavenged and recycled and results in more effective retention by the body (Malbe et al., 1995). The replacement of sulfur with Se in methionine also increases the animal's tolerance for higher concentrations of Se without having a toxicity problem (Spears and Spears, 2003). Because selenomethionine is better absorbed by ruminants than inorganic Se supplements, lower supplementation amounts could still ensure adequate Se supply to the animal. This could offer a more efficient and economical Se delivery system for the producer. Spears (2003) reported that the incorporation of Se as a protein increases Se concentration in the tissue and milk of ruminants and there is less excretion of Se through urine when selenized yeast is supplemented compared to sodium selenite. Spears (2003) found that absorption of orally supplemented sodium selenite was only 34% compared to 85% absorption when organic Se was used in swine.

Selenium tends to accumulate in hierarchy order of kidneys, liver, heart and then skeletal muscle (Taylor et al., 2008). When diets in finishing feedlot steers were supplemented with Se-enriched wheat (selenomethionine) at 9 times the recommended Se concentration, steers had a significantly higher Se accumulation in the semitendinosus muscle compared to steers supplemented with inorganic sodium selenite at the same concentration (Lawler et al., 2004). Even though the diets were formulated to deliver supranutritional concentrations of Se, no negative side effects from the high concentration of Se were observed. This confirms earlier suggestions that delivering elevated concentrations of Se in ruminant diets may not result in toxicity issues. Other studies have confirmed increases in muscle Se accumulation through the use of selenized yeast products. Juniper et al. (2008) reported a 48% increase in longissimus muscle (LM) Se retention when organic selenized yeast was supplemented compared to inorganic sodium selenite. Kidney and liver Se concentrations were also increased with the use of Se-yeast. These findings were similar to the results found in lambs fed inorganic and organic sources of Se. Lambs supplemented with selenized yeast had 56% increase in the LM as compared to lambs fed the same concentration of sodium selenite (Vignola et al., 2009). Beef products are one of the most bioavailable sources of Se in American diets. In areas where Se is lacking in the human diet, Se-enriched beef from ruminants fed high concentrations of Se-yeast could be beneficial.

### 1.6 Selenium and Carcass Quality

Many cattle in the U.S. are sold on a carcass adjusted basis. Improving carcass characteristics and yield grade offer a price incentive for feedlot operators. Meat quality, flavor, and lipid stability are major components of the value of that product. Oxidative damage to lipids occurs in the living animal due to an imbalance between free radicals and antioxidants as discussed earlier (Morrissey et al., 1998). Lipid oxidation leads to discoloration, off-odors, and poor flavors in the meat (Morrissey et al., 1998; Grady et al., 2001). Proper antioxidant defense while the animal is alive can help to partially alleviate some of the oxidation reactions that can occur during handling, processing and storage of the carcass (Morrissey et al., 1994). Juniper et al. (2008) was able to increase GPx activity in the muscle of 20-month old steers by feeding Se-yeast compared to steers not supplemented with any Se. Measurements taken 0 and 10 days postmortem showed a significant increase in tissue GPx activity; however, they were unable to correlate this to increased shelf-life for the final product (Juniper et al., 2008). In agreement, when supplementing high concentrations of Se as high-Se wheat, Taylor et al. (2008), found that Se concentration and GPx activity in the kidneys, liver, and foreshank increased compared to steers fed normal wheat grain, but no change was observed in any other nutrient or in any display-life attribute.

Although Se was fed at 3 times the legal limit enforced by the FDA, again there were no negative side effects observed. Both studies confirmed increases in Se availability in the meat from the steers on the high Se-yeast treatment, which could benefit humans consuming this product in areas where a Se deficiency exists.

### 1.7 Developmental Programming

One area of interest and application in ruminant animals is the idea of affecting long-term growth of progeny by altering maternal nutrition during gestation and/or lactation, often referred to as developmental programming. The effect of maternal supplementation of Se on progeny growth development has not been extensively investigated. Selenium is known to readily cross the placenta and to also enter the mammary gland (Pehrson et al., 1999), therefore, increasing or decreasing Se availability to the cow will have a direct effect on the Se status of the fetal and neonatal calf (Grace and Wilson, 2002). Maternal supplementation of Se could decrease the incidence of white muscle disease in progeny, particularly in areas of low soil Se concentration. Improving the Se status of the cow may also decrease the risk of the offspring suffering from subclinical Se deficiency symptoms such as decreased growth and immune function.

The concept of developmental programming originated from human epidemiological studies that linked low birth weight and poor maternal nutrition to increased incidences of metabolic disease of the progeny as adults (Barker et al., 2002). Recent research in livestock has concluded that the maternal diet has an extended long-

term effect on production characteristics in the offspring (Wu et al., 2006). Funston et al. (2010) defined developmental programming in livestock as the theory that a “maternal stimulus or insult” at a critical period during fetal development has long-term effects on the offspring. Developmental programming can also be extended to the lactation period, as there are critical periods of development that occur early on in the post-natal period (Funston et al., 2010). The impacts of developmental programming have been very broad. Maternal treatments have ranged from nutritional restriction to nutritional excess of nutrients such as protein, energy, and minerals in excessive amounts. Both under and over-feeding the dam can restrict nutrient flow to the progeny (Vonnahme et al., 2003; Wallace, 2004) and result in poor growth.

Changes in the DNA sequence are not responsible for developmental programming, rather, changes in how the genes are expressed are responsible (Gicquel et al., 2008; Maccani and Marsit, 2009), a concept often referred to as epigenetics. Alterations in epigenetic expression of the embryo’s genome is influenced by the external environment of the growing embryo (Gicquel et al., 2008). There are four main modes of epigenetic regulation: DNA methylation, imprinting, RNA mediated control, and histone modification (Maccani and Marsit, 2009). During embryo development there are several resetting periods of the germ-line and any external influence during these times could have a long-lasting effect on the life of the developing embryo (Maccani and Marsit, 2009). Imprinting of the placental tissue also offers a key period when external influences can affect the embryo. Allelic expression is exaggerated in the

placenta and imprinting alterations can lead to several pathologies of the placenta such as; intrauterine growth restriction and pre-eclampsia (Maccani and Marsit, 2009).

Changes in epigenetic expression are not restricted to fetal development but are also influenced by postnatal nutrition and environment (Dolinoy et al., 2007; Gicquel et al., 2008). While examining monozygotic twins, Fraga et al. (2005) found that epigenetic markings have the ability to accumulate with age. However, alterations earlier on in embryo development tend to have the greatest impact due to the few number of cells that multiply and provide the template for the majority of the adult (Feil and Fraga, 2012). Epigenetic markings are also stable in somatic cells which is important since these modifications could play a role in developmental programming (Dolinoy et al., 2007).

#### 1.7.1 Timing of Maternal Supplementation

Timing of supplementation during gestation has been key to the appropriate effects of nutritional adjustments. In most ruminant production systems there are several different opportunities where nutritional stress could take place due to increased demands on the dam. These include the stages of fetal and neonatal development when the needs of the fetus/neonate are in competition with the needs of the dam. For example, in a pregnant heifer that still needs to grow or during milk production when nutritional requirements are split between fetal development and milk synthesis (Wu et al., 2006). Poor quality feed and/or decreased feed availability can also justify feeding of supplemental nutrients. These include late summer when forage has

reached maturity or during a drought that results in decreased quantity of feed, or in the winter when animals are consuming poor quality forage. In the Midwest, late gestation in spring calving herds tends to be during the winter months when most maternal supplementation takes place. If the harvested or grazed forage is being fed is of low quality and is coupled with the increasing nutrient demand of the gravid uterus and developing fetus, fetal development could be negatively affected (Vonnahme et al., 2003). The majority of fetal growth occurs during the last 100 days of gestation and any maternal nutrient supplementation or restriction in this time frame can significantly affect fetal development (Greenwood and Cafe, 2007; Gunn et al., 2014). Decreased BW can result from nutritional restriction in the dam, whereas improper metabolic activity in the calf can result from excess nutrition to the dam during gestation and lactation

The stage of gestation when supplementation is altered can determine the physiological or pathological effect that can take place in the offspring. There are four stages at which nutritional restriction and/or supplementation effect fetal development: conception, and early, mid, or late gestation with each stage effecting different aspect of development (Du et al., 2010). Specifically, these four stages are associated with ovulation, placental growth and organogenesis, myogenesis, and adipogenesis, respectively. The hippocampus-hypothalamo-pituitary-adrenal (HHPA) axis is more responsive during late gestation. As a result, supplementation during this time has a greater effect on muscle growth (Gicquel et al., 2008). Although the demand for nutrients by the fetus are highest during late gestation, the fetal growth trajectory is

determined by its genetics and any environmental effects that occurred during early gestation (Barker, 2012). Thus, many metabolic disorders are a result of poor nutrition during early gestation. According to Symonds et al. (2010) actual muscle, tissue, and organ development takes place during early gestation (1-3 months) however, the increase in actual tissue mass is more proficient during late gestation ( $\geq 210$  d) (Symonds et al., 2010). Lambs fed at either 68.1% and 86.7% their maintenance energy from early to mid gestation showed no effects on fetal weight (Zhu et al., 2007). A change in adipose deposition was correlated to alterations in their metabolism (Bispham et al., 2015). As a result, this could increase the risk of glucose intolerance and insulin resistance later on in life for the offspring (Godfrey and Barker, 2000).

Partitioning of nutrients to the fetus is based on a hierarchy that focuses on the immediate survival of the fetus. This hierarchy is led by the brain, while organs not critical to the immediate survival, such as the kidneys and lungs, are at the bottom (Barker, 2012). In livestock production, this is often seen as compensatory growth. After an adversity has been overcome, the growth trajectory is met through an acceleration in growth. This therefore ranks muscle then fat at the bottom of the hierarchy of nutrient partitioning. Effects of maternal supplementation on offspring development have been shown with lambs. Lambs from nutrient restricted dams during mid- and late-gestation had increased fat and decreased muscle development (Zhu et al., 2006). In studies comparing the effects of pre-calving versus post-calving maternal nutrition, Hight (1968) found no difference in calf progeny weaning weights for cows fed a high plane of

nutrition during gestation and lactation compared to cows fed a low plane of nutrition during gestation and a high plane of nutrition during lactation. This was attributed to increased energy demand during lactation for the cow (Hight, 1968). According to Greenwood and Cafe (2007), after birth and prior to weaning, milk amount and composition have the most significant effect on calf growth. Milk is relatively high in glucose, amino acids and fat, which directly affects the development of the digestive, metabolic, and endocrine systems of the progeny (Greenwood and Cafe, 2007).

These noted effects on offspring development due to maternal nutrition have led to question of how adjusting mineral supplementation to cows can affect the mineral status and gene expression in the suckling calf. In particular, the efficacy of the placental transfer of inorganic mineral products compared to organic mineral products may have the potential to influence progeny development.

#### 1.7.2 Selenium Source Effect on Progeny Status

Maternal supplementation of Se-yeast to lactating cows was shown to increase placental transfer of Se to calves (Pehrson et al., 1999). Pehrson et al. (1999) examined the effects of Se-yeast and sodium selenite minerals in free choice supplementation during late gestation and early lactation on cow and calf performance. Although there were no significant differences in either Se status or GPx activity of the cow, there was a significant increase in whole blood Se content, plasma Se content, and GPx activity of the calf (Pehrson et al., 1999). Whole blood and plasma Se concentrations decreased throughout the trial for calves from cows supplemented with sodium selenite. In that

study, it was observed that some calves even fell below 50 ng/ml of whole blood Se and were at risk for nutritional myodegeneration in the selenite treatment. However, there was an increase in the Se status for both plasma and whole blood in calves from cows fed the Se-yeast treatment (Pehrson et al., 1999). Elevated Se status of the calf was attributed to increased Se transfer through the placenta and increases in the Se concentrations in the cow's milk when Se was supplemented as Se-yeast product. The increased buildup of Se concentrations in the calf resulted in increased antioxidant defense and immune function through elevated GPx activity. The results concluded that offering Se free choice at 30 mg of Se/kg in the form of Se-yeast would provide enough Se for both the cow and calf, however the same amount would not suffice in the form of sodium selenite.

The increased transfer of Se across the placenta to calves could result in increased Se status, immune function, and growth of calves. Ammerman et al. (1980) reported that feeding Se enriched linseed meal to beef cows from mid gestation through early lactation increased calf tissue Se concentrations compared to calves from cows supplemented with sodium selenite. The same study demonstrated that maternal supplementation of an organic sources of Se during gestation had a beneficial effects on post-weaning growth and average daily gain of the offspring, despite a lack of Se supplementation in the offspring's diet (Ammerman et al., 1980). In agreement, Gunter et al. (2013) observed that calves from cows supplemented with Se-yeast had elevated whole blood Se content and GPX erythrocyte activity compared to calves from cows

supplemented with inorganic Se even though no direct supplementation was offered to the calves. Guyot et al. (2007) demonstrated in grazing, gestating cows offered a free-choice mineral, a significant elevation in the whole blood Se content of both the cows and their calves was observed when they were supplemented with 0.5 mg/kg Se as Se-yeast as compared to 0.5 mg/kg Se as sodium selenite, which led to an increase in the GPx activity of the calves. This was in agreement with previous work performed by Malbe et al. (1995), where cows supplemented with Se-yeast during gestation and lactation had an increase in whole blood Se concentrations and GPx activity compared to cows supplemented with selenite. Cows supplemented with Se-yeast also had higher concentrations of Se in their milk and produced calves with improved ADG compared to cows supplemented with sodium selenite (Guyot et al., 2007). Improved ADG was attributed to decreased oxidative breakdown in the tissue of the calves. Other reports have shown that increased availability of Se to calves can boost body weight gains (Wichtel et al., 1996). Wichtel et al. (1996) also reported that there may be an interaction between iodine and Se in thyroid metabolism when Se is short in supply. Type 1 Iodothyronine-5'-deiodinase is a Se dependent enzyme and convert thyroxine to the more active thyronine. This is the major deiodinase found in the liver, kidney, and skeletal muscle. Without proper Se availability a biologically inactive form of triiodothyronine is produced resulting in issues with hyperthyroidism, leading to poor growth and development. If proper dietary concentrations of Se are provided, production of triiodothyronine can take place (Wichtel et al., 1996) However, work relating improved growth directly to Se availability is limited.

Elevated GPx activity in the calves, if maintained, could help to improve weaning performance. Gunter et al. (2003) observed that if gestating cows were supplemented with sodium selenite, whole blood Se concentrations in their calves were just slightly above what would be considered a deficiency concentration. Lambs from ewes supplemented with 81.8 µg/kg of BW of Se-yeast from early through late gestation had improved average daily gain and gain:feed compared to lambs from ewes supplemented with inorganic Se 81.8 µg/kg of BW (Neville et al., 2010). These positive results were attributed to decreased oxidative damage and increased vascularity and crypt depth in the gastrointestinal tract of feedlot steers supplemented with Se-yeast, which in turn increased jejunal mass and resulted in increased metabolic activity and nutrient absorption (Soto-Navarro et al., 2004). Ward et al. (2008) also reported increased Se transfer from ewes to their lambs that increased the lambs gastrointestinal mass and body mass near term. Therefore with the increased Se transfer noted in cows supplemented with organic Se to their calves, the Se status of these calves while in the feedlot could remain elevated, thus improving their performance through slaughter.

### 1.7.3 Selenium Source Effect on Milk

After birth and prior to weaning, milk composition and production of the cow has the greatest effect on calf health and performance (Greenwood and Cafe, 2007). By increasing the Se concentration in milk, the amount of Se being delivered to the calf can be increased, improving calf health. However, different forms of Se are transferred into milk at different rates. Ortman and Pehrson (1999) observed that Se-yeast increased

milk Se 130% compared to cows not supplemented with Se, whereas supplemental selenate and selenite only increased milk Se 20% compared to control cows. In this study, supplemental Se ranged between 0.24 and 0.31 mg/kg DW while the control group of cows remained unsupplemented. A substantial increase in Se status of the calf was also noted for cows supplemented with selenized yeast (Ortman and Pehrson, 1999). Other studies (Conrad and Moxon, 1979; Malbe et al., 1995) have reported similar results. Guyot et al.(2007) reported that when cows were supplemented (0.5 µg/kg) with Se-yeast or sodium selenite, milk Se concentrations were greater in Se-yeast fed cows . This led to improved plasma Se and average daily gain in calves from cows supplemented with Se-yeast compared to calves from inorganic Se cows (Guyot et al., 2007).

Improving the Se status of the cow has also increased the Se status of the suckling calf. After supplementing late gestation and early lactation Hereford cows with varying forms of Se (selenate, selenite, and Se-yeast), researchers in Sweden found increased whole blood and plasma Se concentrations in calves nursing cows supplemented with Se-yeast compared to calves nursing cows supplemented with selenate or selenite at 0.24 to 0.31 mg/kg DM of Se (Ortman and Pehrson, 1999). Cows supplemented with Se-yeast had 60% higher whole blood Se concentrations compared to cows not supplemented with Se while cows supplemented sodium selenite and selenate had a whole blood Se content that was only 35% higher compared to non-supplemented control cows. Plasma and milk Se concentrations were also elevated for

cows fed Se-yeast compared to cows fed inorganic Se. This demonstrated improved absorption of organic Se compared to inorganic Se. Although differences in whole blood and plasma concentrations were significant, there was no effect on the concentration of GPx activity of the cows (Ortman and Pehrson, 1999).

### 1.8 Feedlot Performance

No one has yet to follow calves from organic Se supplemented cows through the feedlot and compared them to calves from cows supplemented with inorganic Se. Decreased oxidative damage and improved immune function can be key when animals are weaned and transitioned onto a feedlot finishing diet. The majority of disease outbreaks, morbidity, and digestive issues in the feedlot take place within the first 21 days of the animals arrival in the feedlot (Kelly and Janzen, 1986). Maternal supplementation with selenized yeast products could allow progeny that enter the feedlot to have improved vaccine responses, dry matter intake, and decreased morbidity. Ensuring that newly received animals have appropriate Se status prior to entering the feedlot could potentially provide a large monetary benefit by cutting death loss, increasing weight gain, and decreasing treatment cost in these animals. Furthermore, ethanol by-products are an essential part of the diet in Midwestern feedlots. However, sulfur is known to fluctuate in products like dried distiller grains (DDG). Researchers from South Dakota State observed a range from 0.36% to 0.69% sulfur in DDGS from different ethanol plants (Holt and Pritchard, 2004). The NRC (2000) reports that concentrations of 0.40% sulfur could lead to decreased performance in

cattle. Sulfur is known to bind up Se and decrease its availability to livestock (Wichtel, 1998). High concentrations of sulfur can also decrease intake, which could compromise Se intake. Feedlots in the Midwest that receive cattle that have been grazing areas already low in Se may be able to benefit greatly from a readily available source of Se in the feedlot diet. This could help decrease some of antagonistic effects that sulfur can have on Se absorption.

### 1.9 Summary

The availability of Se is key to the health and performance of ruminants. When proper concentrations of Se are maintained, animals experience improved immune function, growth and fertility. Because of the large variation in soil Se content and resulting plant Se concentration variation in the US, it is a common practice to supplement livestock with Se. Traditional forms of inorganic Se such as sodium selenite are reported to have decreased absorption when exposed to the natural reducing environment of the rumen. This has led to increased use of organic Se-yeast products that contain selenomethionine as a source of Se. Selenomethionine has been shown to have increased bioavailability, increasing the retention of deficient and/or adequate Se and increasing the tolerance for excess Se in ruminants. This offers an economical benefit to producers by allowing them to supplement lower concentrations of the mineral to their livestock while still maintaining proper Se status. Selenomethionine has also been shown to transfer more readily through the placenta and into milk compared to inorganic Se which increases the Se status of the offspring. Increasing the Se status of

the dam in late gestation and early lactation may program the progeny for increased health and growth throughout their life. It may also limit the amount of supplementation that must be provided to the offspring post-weaning.

Although a large amount of work has been performed showing the benefits of supplementing both maternal and growing ruminants with Se-yeast as compared to inorganic compounds, there has been little focus on examining the effects of maternal supplementation on offspring performance following weaning. Increases in bioavailability in Se-yeast products have been demonstrated to increase ruminant absorption and retention of Se which can lead to increased oxidative defense, weight gains, and feed efficiency in growing ruminants. Increased Se availability in progeny may improve immune function during times of stress, such as the transition period during the first three weeks in the feedlot. This is where the majority of treatments and death loss occur on American feedlots. Studies also show that these animals have elevated tissue Se concentrations which could have the potential to limit some lipid oxidation post-slaughter and improve shelf stability. Furthermore, Selenium-yeast products have been shown to increase tissue and organ accumulation in feedlot steers compared to supplementation with inorganic Se. Beef is one of the most bioavailable Se source for human nutrition. By increasing Se muscle content in feedlot steers, there is potentially an increase in the Se availability to the human diet.

The increase in maternal Se transfer from gestating cows supplemented with Se-yeast to their calves has led us to believe that supplementing gestating and lactating

cows with Se-yeast could increase the Se status of the cow and her offspring throughout the pre-weaning and post-weaning phases of life. Increased Se status of the progeny should in turn increase the animal's antioxidant defense, and improve growth, performance, and carcass quality during the finishing phase without additional supplementation.

CHAPTER 2 EFFECTS OF ORGANIC OR INORGANIC SELENIUM SUPPLEMENTATION  
DURING LATE GESTATION AND EARLY LACTATION ON COW AND PRE-WEANING  
CALF PERFORMANCE

2.1 ABSTRACT

Angus x Simmental cows (n=48, BW = 594 kg, BCS = 5.26, Age = 2.7), pregnant with male fetuses, were used to determine the effect of selenium (Se) source during the last 80 d of gestation and first 105 d of lactation on cow and calf performance. At 203 d in gestation, cows were randomly allotted to 1 of 3 treatments and blocked by body weight, breed composition, and calf sire: organic Se, inorganic Se, or no Se. Diets contained corn silage, corn stover, haylage, DDGS, and mineral and were formulated to contain 10.4% CP and 0.90 Mcal/kg NE<sub>g</sub> during gestation and 12.1% CP and 1.01 Mcal/kg NE<sub>g</sub> during lactation. Diets were fed daily as a total mixed ration and none, 0.30 mg/kg Se as sodium selenite, or 0.30 mg/kg Se as Sel-Plex<sup>®</sup> were top-dressed daily. Treatment diets were fed through 105 d post-partum (DPP). At 68 DPP milk production was calculated using the weigh-suckle-weigh procedure and a milk sample was collected to determine composition. At 105 DPP cow-calf pairs were commingled until weaning at 210 DPP. Cow BW and BCS ( $P \geq 0.56$ ) did not differ between treatments at any time point during the study. Milk production, milk fat, and total solids ( $P \geq 0.38$ ) did not differ among treatments. Milk protein tended to increase in cows fed inorganic Se

compared to cows fed organic Se ( $P = 0.07$ ) and milk lactose tended to be greatest in cows fed organic Se ( $P = 0.10$ ). Conception to AI and overall pregnancy rates did not differ between the diets ( $P \geq 0.39$ ). Calf weights and ADG did not differ through 105 DPP ( $P \geq 0.77$ ) or for the entire pre-weaning period ( $P \geq 0.33$ ). Plasma Se concentration did not differ between treatments for the cows ( $P \geq 0.37$ ). Liver Se concentration in cows fed either inorganic or organic Se treatment were greater than control cows ( $P < 0.01$ ). There were no treatment effects on calf plasma Se concentration ( $P \geq 0.90$ ). Muscles biopsies taken at 105 DPP also were not different ( $P = 0.45$ ). In conclusion, dietary Se source did not affect cow performance, milk production, or reproductive ability. Organic Se decreased milk protein and increased milk lactose, but did not alter pre-weaning progeny performance.

## 2.2 INTRODUCTION

The primary source of Se for grazing animals is provided by plants, which assimilate soil Se into their tissue. However, the amount of soil Se is highly variable throughout the country. Soils in the Midwest and Southeast tend to be deficient in Se, while regions of the Great Plains tend to be adequate, with some locations reaching toxic concentrations of Se (Dargatz and Ross, 2014). Livestock raised in areas of the southeast and Midwest commonly suffer from mild to severe Se deficiency, whereas livestock in the Great Plains can suffer from Se toxicity. It is common practice throughout the United States to supplement cow herds with selenium in their mineral

programs, and because of the variability in soil Se concentration, supplementation is limited to 3.0 mg/d by the FDA.

The traditional form of Se supplementation is sodium selenite, which is an inorganic Se salt product. Research has demonstrated that the natural reducing nature of the rumen decreases the absorption of selenites (Wright and Bell, 1966). Organic Se is chelated to an organic molecule, most typically an amino acid such as cysteine or methionine. Selenium found in forages and grains is typically in an organic form, which is absorbed and deposited in tissues by the animal as an amino acid. Chelation to an organic molecule increases absorption and retention of Se and can increase an animal's tolerance for higher concentrations compared to many inorganic products. Organic Se products are produced commercially by growing yeast in Se-enriched environments, hence the term Se-yeast. Selenized yeast is absorbed as selenomethionine, which increases Se bioavailability. Once consumed, selenomethionine is incorporated into the body as the amino acid methionine which increases tissue deposition of Se. Many studies have shown increased blood and tissue Se concentrations when Se-yeast products were used as a Se source (Pehrson et al., 1999; Guyot et al., 2007; Gunter et al., 2014).

It has recently become clear in humans that optimal synthesis of some selenoproteins require higher amounts of dietary Se than what is currently recommended (Xia et al., 2005). Because L(+)-selenomethionine contained in Se-yeast is nonspecifically incorporated into tissues in place of methionine, higher Se assimilation

occurs in tissues and milk and thereby provides animals and their progeny with increased Se (Gunter et al., 2003). In fact, supplementation of supranutritional concentrations of Se to pregnant ewes has been demonstrated to increase fetal placental tissue (Lekatz et al., 2010) and mammary gland vascularity compared with ewes supplemented to meet their Se requirement (Jorgenson et al., 2010; Vonnahme et al., 2011). Nutrient exchange is related to blood flow, thus an increase in fetal placental vascularity due to supplementation of supranutritional concentrations of Se to pregnant ewes is thought to be responsible for increased progeny birth weights (Reed et al., 2007; Meyer et al., 2010; Vonnahme et al., 2010). In addition, increased vascularity in the mammary gland due to supranutritional supplementation of Se results in greater milk production with increased concentrations of fat and protein (Swanson et al., 2008; Meyer et al., 2011), increased neonatal growth, and increased fat tissue in progeny compared with adequate supplementation of Se (Vonnahme et al., 2010). While it is clear that supplementation of supranutritional concentrations of Se to ewes impacts fetal and neonatal growth and body composition in sheep, the effects of organic vs. inorganic Se supplementation of beef cows on progeny growth, carcass composition, and gene expression, particularly vasoactive, angiogenic, and selenoprotein gene expression is less clear because limited data exist.

The developmental responses in progeny of mothers supplemented with supranutritional concentrations of Se appears to be specific to organic forms of Se. Guyot et al. (2006) reported that feeding pregnant cows 0.5 mg/kg Se as Se-yeast

increased calf performance compared with feeding pregnant cows 0.5 mg/kg Se as sodium selenite. Increased Se transfer to offspring of ewes programs the progeny for increased gastrointestinal and body mass near term (Reed et al., 2007). These developmental changes have long-term impacts on performance. For example, when progeny were taken to maturity, it was demonstrated that supranutritional maternal Se increased progeny ADG compared with adequate maternal Se, although DM, OM, ADF, NDF, and N digestibility were decreased (Neville et al., 2010b). These long-term changes appear to be due to prevention of oxidative damage and increased vascularity in the gastrointestinal tract, since supranutritional Se increased intestinal mass and total proliferating crypt cells in feedlot steers (Soto-Navarro et al., 2004).

Development of a robust antioxidant defense system is critical for producing healthy and efficient offspring and for maintaining profitability of a cow-calf enterprise. Both prenatal and postnatal Se supplementation are essential for the offspring antioxidant system. Prenatal Se supplementation provides an effective antioxidant system that is already in place at the time of birth and postnatal Se supplementation becomes the main determinant of progeny Se status the first few days after birth as demonstrated by Gunter et al. (2003) in beef cattle progeny. Thus feeding a source of highly available Se during the last trimester of pregnancy, a time when supplemental feed is typically fed, could be a practical time-frame for producers to feed Se-yeast. Further, data from our laboratory demonstrates that nutritional intervention during the last trimester of gestation and first 100 d of lactation significantly impacts subsequent

performance of offspring (Gunn et al., 2011, 2012a,b). Thus, our hypothesis was that inclusion of organic selenium in gestating and lactating beef cow diets will improve selenium status of cows and their progeny, as well as pre-weaning growth of male progeny compared with inclusion of inorganic Se. Our objective was to quantify the effects of organic and inorganic selenium on late gestation and early lactation cow productivity and characterize changes in pre-weaning performance and selenium status in male progeny associated with developmental programming.

## 2.3 MATERIALS AND METHODS

### 2.3.1 Cow Diets and Treatments

The study was performed at the Purdue Animal Sciences Research and Education Center in West Lafayette, IN. All procedures and protocols followed guidelines in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010) and were approved by the Purdue Animal Care and Use Committee. Angus x Simmental cows ( $n = 48$ ,  $BW = 594 \text{ kg} \pm 5.6$ ,  $BCS = 5.26 \pm .119$ ,  $\text{Age} = 2.3 \pm .06$ ), confirmed pregnant with male fetuses, were used to determine the effects of supplemental selenium (Se) source during the last 80 d of gestation and first 105 d of lactation on cow performance, milk composition and pre-weaning calf growth.

At 203 d of gestation, cows were blocked by BW, age, and calf sire and then randomly allotted to 1 of 3 treatments. Treatments consisted of no supplemental Se, 3 mg/d inorganic Se (sodium selenite), and 3 mg/d organic Se (Sel-Plex®, Alltech Inc.,

Nicholaville, KY). Although the control cows had no direct Se supplementation, there was a basal concentration of 0.84 mg/kg of Se provided. Cows were placed in an open-sided barn with bedded, individual pens (2.4 x 9.1 m) over a concrete floor, and fed a gestation ration consisting of 63.0% corn silage, 22.4% corn stover, 8.2% grass haylage, 6.2% dried distillers grains with solubles (DDGS) and 0.23% supplement (Table 1.1). Gestation diets were formulated to deliver 10.4% crude protein and 0.90 Mcal/kg NEg. At calving, cows were then supplied 39.0% corn silage, 21.2% corn stover, 7.6% grass haylage, 10.1% DDGS, 22% soybean hulls (SBH) and 0.23% supplement to provide 12.1% crude protein and 1.01 Mcal/kg NEg during lactation. Diets were designed to meet or exceed protein, energy, mineral, and vitamin requirements (NRC, 2000). Both diets were formulated using the individual ingredient wet chemistry analysis (AOAC, 1990) prior to the beginning of the trial (Sure-Tech Laboratories, Indianapolis, IN). Basal diets provided 0.07 mg/kg and 0.11 mg/kg of Se for the gestation and lactation diets, respectively. Diets were delivered as a total mixed ration (TMR) once daily at 0800 h in concrete bunks. Selenium supplements were top-dressed immediately after feeding at a rate 0.454 g/Kg of dry matter (DM) per head. The top-dress contained DDGS, vitamins, minerals, and Se to achieve a Se concentration of 0, 3.0, and 3.0 mg/d in the control, inorganic, and organic Se treatments, respectively. Feed samples were collected and composited bi-weekly and oven-dried at 60°C for 72 h for DM determination. Dried feed samples were ground using a standard Wiley laboratory mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA), and composited at the end of the experiment for analysis of CP (micro-Kjeldahl N x 6.25), NDF and ADF using an Ankom<sup>200</sup> Fiber Analyzer (ANKOM

Technology Corporation, Fairport, NY), ether extract (method 920.39; AOAC, 1990), and minerals (Ca, P, Mg, K, S; method 968.08; AOAC, 1990). As-fed formulations were adjusted for DM content accordingly every other week.

Dietary treatments were terminated at 108 d post-partum (PP) and cows and calves were then placed on summer pasture and managed as one group, without supplemental selenium, until weaning at 216 DPP. Initial and final BW were determined by averaging pre-prandial weights on two consecutive days. Subsequent BW and BCS (1 = emaciated, 9 = obese; Wagner *et al.*, 1988) were assessed monthly throughout the treatment period. Body condition scoring was conducted by the same investigator at all time points throughout the study. A BW for cows was taken when approximately 50% of the cows had calved (day 83). Weight of cows that had calved on d 83 of the study was adjusted to a pregnant basis using the gravid uterine weight calculation (Ferrel, 1976) for determination of gestation period data. Conversely weight of cows that were pregnant on d 83 of the study was adjusted to a non-pregnant basis for determination of lactation period data using the same equation. Cow weights were also assessed at weaning (216 dpp) to aid in interpretation of cow performance between the end of the study and weaning.

### 2.3.2 Milk Analysis

Milk production was measured on d 68 PP using a 12 h weigh-suckle-weigh procedure (Buskirk *et al.*, 1992). Calves were separated from their dams at 0000, allowed to nurse at 0600, and then separated again until 1200. At 1200 calves were

weighed prior to nursing and re-weighed once nursing had stopped. The difference in calf weight before and immediately after nursing was used to calculate milk production during the 6 h period. The weigh-suckle-weigh procedure was then repeated at 1800. Milk production at 1200 and 1800 were added together then multiplied by two to estimate the milk production over a 24-hour period. During separation cows were returned to their pens where they had access to both feed and water while calves were grouped in pens of 4 to 5 without access to water or feed until the end of the procedure.

Before feeding on 62 DPP, calves were separated from cows for 3 h and a milk sample was totally collected from one quarter of each cow by hand-milking. After collection, milk was placed in a vial containing methylene blue and shipped to Dairy One Cooperative (Ithaca, NY) for analysis of protein, fat, lactose, total solids, and milk urea nitrogen (MUN). Milk samples were also stored for later Se analysis in a -20 °C freezer. Samples of milk were thawed in a water bath at 50°C for 30 minutes and 0.5 g subsamples in duplicate were digested with a 4:1 ratio of nitric acid and perchloric acid, followed by derivatization with EDTA and diaminonaphthalene solutions. A cyclohexane extraction was then performed and samples were transferred into a white well plate (Dynex Technologies Inc., Chantilly, VA). Samples were read in a fluorometer with excitation wavelength at 375 nm and emission at 525 nm (Magellan GENios Pro, Tecan Trading Ag, Switzerland).

### 2.3.3 Estrus Synchronization and Breeding

Cows were synchronized into estrous using the 5-day Co-synch + CIDR protocol and time artificially inseminated (TAI) in May of 2013. The 5 d Co-Synch + CIDR protocol consisted of insertion of an intravaginal progesterone insert (CIDR, Pfizer Animal Health, New York, NY) concurrent with administration of 100 µg of GnRH (Fertagyl, Intervet/Schering-Plough Animal Health, Summit, NJ) at protocol initiation. Five days later, the CIDR was removed and 25 mg of PGF<sub>2α</sub> (Lutalyse, Pfizer Animal Health, New York, NY) was given at CIDR removal and again 8 h later. Seventy-two h after CIDR removal and initial PGF<sub>2α</sub> injection, all cows were time artificially inseminated (TAI) concurrent with GnRH administration (Fertagyl; 100 µg). Ten days after TAI, the experimental period ended, cows from all treatments were commingled, and were placed with a bull for the remainder of the 60 day breeding period. Pregnancy was confirmed using ultrasound (Variable MHz linear array transducer, MicroMaxx, Sonosite, Bothell, WA), 30 and 90 days post-AI to determine conception and overall pregnancy rates.

### 2.3.4 Pre-Weaning Calf Performance

Calf weights were recorded at birth and on the same day as cows and were used to calculate calf ADG. At calving, scores were given for calving ease (CE) and calf vigor (V) and calf birth weight was recorded. Cows were given 1 hour after the initiation of hard labor to deliver the calf before assistance was given. Calving ease was scored on a scale of; 1 = no assistance; 2 = easy pull; 3 = mechanically assisted pull; 4 = abnormal

presentation; 5 caesarian-section. Calf vigor scores followed the subsequent ranking: 1 = nursed on own immediately; 2 = nursed on own, but slow to begin; 3 = required assistance to nurse; 4 = died soon after birth. Cow udder scores were also given at calving based on the following scale; 1 = ideal; 2 = not ideal, but calf nursed on own; 3 = calves may require intervention; 4 = worst case. At 216 DPP, calves were separated from cows and weaned. Two-day weights were taken and averaged together to calculate weaning weight for the calves. Calves were placed on pasture for 28 d for the weaning process and adapted to the feedlot diet.

#### 2.3.5 Plasma, Liver, and Muscle Se

Blood was collected from both cows and calves every 28 d throughout the study prior to daily feeding. Blood was collected through jugular venipuncture using 10 ml Vacutainer tubes containing 158 USP Sodium Heparin (Becton Drive, Franklin Lake, NJ). Blood was inverted and placed on ice until centrifugation at 3,000 x g for 20 minutes at 4°C. Plasma was separated and stored at -20°C until analysis for Se content. At the termination of the study (105 d PP), liver biopsies were taken from cows using the blind aspiration technique (Greenfield et al., 2000). Briefly, a 0.5 g sample was collected, rinsed with phosphate buffered saline, placed in a tube and flash frozen in liquid nitrogen. Samples were stored at -80°C for subsequent analysis of Se (Brennan et al., 2011).

Muscle biopsies from the steers were taken at 105 DPP according to the procedures of Pampusch et al. (2008). Steers were restrained in a hydraulic squeeze

chute, hair was removed from the biopsy site, and a local anesthetic (lidocaine HCl; 20 mg/mL; 8 mL per biopsy) was administered. Biopsies were obtained from between the 10<sup>th</sup> and 13<sup>th</sup> rib from alternate sides for sequential samples. The biopsy site was cleansed with Betadine for the initial scrub followed by alcohol, for up to three scrubs. A 1-cm incision was made with a scalpel and a sterile Bergstrom biopsy needle (Stille Surgical, Lombard, IL) was used to obtain 1 g of tissue from the longissimus muscle. The incision was rinsed with sterile saline/water, a topical antibiotic spray was applied and the incision site was covered with a spray-on aluminum bandage. Samples were flash frozen with liquid nitrogen then placed in a -80°C freezer for later analysis of Se content.

#### 2.3.6 Selenium analysis

Muscle (0.5g), and plasma samples (3g) were analyzed for Se content following AOAC procedures (AOAC 996.16). Duplicate samples were digested with a 4:1 ratio of nitric acid and perchloric acid, followed by derivatization with EDTA and diamionaphthalene solutions. A cyclohexane extraction was then performed and samples were transferred into a white well plate (Dynex Technologies Inc., Chantilly, VA). Samples were read in a fluorometer with excitation wavelength at 375 nm and emission at 525 nm (Magellan GENios Pro, Tecan Trading Ag, Switzerland). Liver samples were shipped to Alltech Inc. for Se analysis (Brennan et al., 2011).

### 2.3.7 Statistical Analysis

Timed-Artificial Insemination and pregnancy rates were analyzed using GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC). Cow BW, BCS, milk production and composition, along with plasma and liver Se content were analyzed using the MIXED procedure of SAS. Calf BW, ADG, plasma Se, and muscle Se were analyzed using the MIXED procedure of SAS. The covariance structures autoregressive order one, heterogeneous autoregressive order one, unstructured and compound symmetric were compared and the covariance structure with the smallest Bayesian information criterion was chosen for analysis results. The model included the fixed effects of treatment, day and treatment x day interactions. Animal served as the experimental unit. Simple effects within day were highlighted using the SLICE function of SAS. For all variables analyzed, a  $p\text{-value} \leq 0.05$  was considered significant, while values of  $0.05 > p \leq 0.10$  were identified as a tendency approaching significance.

## 2.4 RESULTS AND DISCUSSION

### 2.4.1 Cow Performance

Cow performance results are presented in Table 2.2. Cow body weights did not differ between treatments throughout the study ( $P \geq 0.70$ ). Selenium source also had no effect on cow ADG, BCS, or DMI ( $P \geq 0.56$ ). No treatment effects were observed for conception to artificial insemination, seasonal pregnancy rates, calving ease, or calf vigor ( $P \geq 0.34$ ). Improved growth has been noted for Se source when cattle began the

study in a Se deficient state (Wichtel, 1998). Gunter et al. (2003) observed an increase in free choice mineral intake in beef cows during the first and last week of the trial when Se-yeast was provided compared to sodium selenite and but did not observe a difference in body weight. A lack of change in body weight when comparing Se sources has been noted by others (Gunter et al., 2003; Kristen M Brennan et al., 2011; Mahima et al., 2012). Vignola et al. (2009) noted no difference in growth rate or feed intake in lambs supplemented with sodium selenite or selenized yeast. Brennan et al. (2011) reported that there was no change in DMI when heifers were provided a diet supplemented with selenized yeast, sodium selenite, or a mix of both. Other research in feedlot steers and heifers found no difference in ADG between selenized yeast and sodium selenite (Richards and Loveday, 2003).

The lack of change in BCS in the current study is consistent with the BCS response reported by Gunter et al., (2003). Variations in cow performance and intake among studies comparing Se sources are potentially due to Se status of the cows at the initiation of the study. In some studies, cows began in a Se deficient state whereas in other studies, cows had adequate Se status. The basal diet in the present study during gestation and lactation contained 0.07 mg/kg and 0.11 mg/kg, respectively. The top-dress for the organic, inorganic, and control treatments contained 2.98 mg/d, 3.05 mg/d, and 0.74 mg/d, respectively. Although the control diet was below NRC recommendations, it could have been high enough to mask differences in performance. Furthermore, vitamin E was included in the diet at 440 IU/kg in the present study. Like

Se, vitamin E is considered a component of antioxidant systems and some reports have found that when livestock are deficient in Se, vitamin E supplementation can benefit the animal's immune system, however these results are not conclusive (Finch and Onn, 1996). According to the NRC (2000), there is no true recommended vitamin E concentration in the diet, however diets usually range between 500 IU/d and 1,000 IU/d. Harrison et al. (1984) reported that the combination of vitamin E and Se decreased the occurrence of retained placentas and uterine infections compared to non-supplemented cows. In a study comparing calves supplemented with Vitamin E in a Se depleted diet to calves supplemented with Se in vitamin E depleted diets, it was noted that subclinical nutritional myopathy was found in both treatments; however, vitamin E supplementation in a Se depleted diet did not improve erythrocyte GPx activity (Walsh et al., 1993)

#### 2.4.2 Milk

Milk production and composition data are presented in Table 2.4. Maternal Se source did not affect milk production, milk fat, total solids, or somatic cell count ( $P \geq 0.38$ ). However, cows supplemented with organic Se tended to have a lower milk protein than cows supplemented with inorganic Se ( $P = 0.07$ ). Milk protein for control cows was intermediate and did not differ from organic or inorganic cows. Cows supplemented with organic Se tended to have higher milk lactose than cows supplemented with either inorganic Se or no Se ( $P = 0.10$ ). There was no effect of Se source on milk production in the current study with an average of 9.30 kg across all

treatments. Although there has been ample work showing the increase in milk Se content through Se-yeast supplementation, very little has focused on the effect of Se source on milk composition and production. Higher milk lactose content usually corresponds with higher milk production but in this case, the organic supplemented cows had the highest % lactose with the numerically lowest milk production, which was unexpected.

Selenium source did not affect milk Se concentrations between the different Se sources ( $P = 0.32$ ). Milk Se concentrations were 22, 33, and 32  $\mu\text{g}/\text{kg}$  for the control, organic, and inorganic supplemented cows, respectively. In contrast, supplementation of Se-yeast has been shown to increase Se concentration in milk and colostrum compared to supplementation with inorganic Se. When dairy cows were supplemented with Se-yeast, their milk Se concentration was roughly twice that of cows supplemented with selenate and selenite (Ortman and Pehrson, 1999). In Se deficient dairy cows, Se-yeast supplementation produced a 3-fold increase in milk Se concentration compared to dairy cows supplemented with sodium selenite (Malbe et al., 1995)

#### 2.4.3 Cow Liver and Plasma Se Content

Cow plasma and liver Se concentration data are presented in Figures 2.2 and 2.3 and Table 2.5. Cows supplemented with organic Se had the highest plasma Se at the start of the trial ( $P = 0.03$ ). However there were no treatment effect on plasma Se throughout the trial ( $P \geq 0.37$ ). Plasma Se concentrations at study initiation were 177,

132, and 116  $\mu\text{g}/\text{kg}$  for the organic, inorganic, and control cows, respectively. Plasma Se concentrations for cows fed the control and organic treatment steadily decreased through 105 DPP, whereas plasma Se for cows fed the inorganic Se treatment remained stable until the last 40 days of the experiment. It is possible that cows built up tissue stores of Se during dietary treatments and depleted plasma concentrations didn't become evident until tissue stores were depleted. The rise in plasma Se during the last 40 d of the experiment coincided with the fall rush of grass, which could explain the elevated plasma Se concentrations. In agreement with the current study, research in gestating and lactating ewes reported a decrease in Se concentration as the study progressed, however selenized yeast still produced greater plasma Se concentrations in cows compared to sodium selenite (Hall et al., 2012). In contrast, supplementation of Belgian Blue cows during gestation with selenized yeast led to a significant increase in plasma Se concentration compared to cows supplemented with sodium selenite (Guyot et al., 2007). Although all groups increased in plasma Se concentration over a 4-week period, cows supplemented with selenized yeast had a significantly greater plasma Se concentration compared to cows supplemented with selenate or selenite (Ortman and Pehrson, 1999).

In general, increases in plasma Se content have been correlated with increased whole blood Se concentration and GPx activity (Ortman and Pehrson, 1999; Juniper et al., 2008; Hall et al., 2012). However, other work has observed increases in plasma Se content with no effects on whole blood Se concentration or GPx activity. Van Ryssen et

al. (1989) observed an increase in blood Se content without any effect on plasma Se concentrations or GPx activity in sheep when Se was provided either as sodium selenite or selenomethionine through high-Se wheat. In addition, gestating and lactating cows given access to the same concentrations of either selenized yeast or sodium selenite had a 25% increase in whole blood Se content, but no difference was observed for either GPx activity or serum Se concentration (Awadeh et al., 1998). In both studies, the lack of change in GPx activity and serum Se concentration was attributed to the nonspecific incorporation of selenomethionine into proteins. In goat dams, which were deemed adequate in Se status, sodium selenite increased GPx activity and whole blood Se to a greater extent than goats supplemented with an organic lactate-protein selenium complex (Pavlata et al., 2011). Although no difference was observed in plasma Se concentrations in the present study, there may have been an increase in whole blood Se content.

Cows from both organic Se supplemented and inorganic Se supplemented groups had significantly greater liver Se concentrations than cows not supplemented at all (381, 368, and 287  $\mu\text{g}/\text{kg}$ ; respectively). The liver plays a crucial role in the metabolism of many trace minerals including Se. Once absorbed, the liver converts Se to selenoproteins, which then re-enter the blood stream (Symonds et al., 1981). Second only to the kidneys, the liver accumulates large amounts of Se and is commonly used to determine Se status of ruminants (Taylor et al., 2008). Cows in the present study that were supplemented with Se (both selenized yeast and sodium selenite) had a significant

increase in liver Se concentration as compared to the control diet. An increase in Se in Se supplemented cows is consistent with other's conclusions (Lawler et al., 2004; Arthington, 2008) and was expected because cows from the control treatment were fed a diet deficient in Se.

Although the liver Se in the cows supplemented with Se were greater than that of the control group in the present study, there was no significant difference between cows supplemented with selenized yeast and those supplemented with sodium selenite. In agreement, Lawler et al. (2004) found no difference between steers supplemented with high-Se wheat (selenomethionine) and those supplemented with sodium selenite. While examining grazing beef cows in highly seleniferous areas versus low seleniferous areas, Hintze et al. (2002) found that the highest kidney and liver Se concentrations were found in animals from low seleniferous areas fed high-Se diets independent of the Se source. This implies that animals are able to develop physiological adaptations that allow them to up-regulate or down-regulate Se absorption (Hintze et al., 2002). In contrast, beef cows supplemented with Se-yeast had greater liver Se concentrations than that of beef cows supplemented with sodium selenite (Arthington, 2008; Juniper et al., 2008). Similar increases in liver Se in selenized yeast vs. inorganic Se have been reported in other species including sheep (Vignola et al., 2009) and finishing pigs (Zhan et al., 2007).

#### 2.4.4 Pre-weaning Calf Performance

Pre-weaning progeny performance is presented in Table 2.6. Calf birth weights did not differ between maternal treatments ( $P = 0.99$ ) and there was no effect of treatment on calf vigor ( $P = 0.34$ ) or calving ease ( $P = 0.62$ ), which can be found in Table 2.3. Throughout the pre-weaning phase of the trial, there were no treatment differences in calf weight ( $P \geq 0.33$ ) or ADG (1.15, 1.17, and 1.14 kg/d for control, organic, or inorganic Se, respectively;  $P \geq 0.83$ ).

Ortman and Pehrson (1999) observed an increase in placental transfer of Se to calves when cows were supplemented with selenized yeast, therefore increasing the Se status of the calf. Furthermore, Conrad and Moxon (1979) concluded that natural and organic sources of Se such as Se-yeast increase milk Se concentration and availability to the calf. During the pre-weaning period, milk is the single most important contributor to the nutritional health of the calf. However, little work has examined the effect that maternal supplementation of different Se sources has on calf growth and health before and after weaning.

Throughout the entirety of the present trial there was not a difference in calf weight or ADG ( $P \geq 0.33$ ). Ammerman et al. (1980) similarly reported no effect on calf weight when sodium selenite was fed in both high-Se diets and low-Se diets to gestating cows and observed no effect when Se-yeast was supplemented to gestating cows versus sodium selenite. Others have also reported no difference between calves from cows

supplemented with either Se source and those not supplemented at all (Phillips et al. 2003; Gunter et al., 2003). In contrast, some reports have found a Se source effect on weight, ADG, and lean:fat ratio in calves whose dams were fed different sources of Se (Spears et al., 1986; Guyot et al., 2007). Spears et al. (1986) reported that when cows fed Se deficient diets received Se and vitamin E injections through gestation and lactation, calves had improved weaning weights and ADG. Belgian Blue calves from Se deficient cows supplemented with Se-yeast during late gestation and early lactation tended to have increased ADG as compared to calves from Se deficient cows supplemented with sodium selenite (Guyot et al., 2007). This increase in growth was attributed to higher milk Se concentration in the cows fed Se-yeast, and the resultant increase in GPx and plasma Se concentrations in the calves from cows fed Se-yeast. However, cows in both Spears et al. (1986) and Guyot et al. (2007) were Se deficient at the start of the study. Cows have the ability to up-regulate and down-regulate Se excretion, absorption, and use based on nutritional status. Therefore cows in the present study were already sufficient in Se, differences in performance due to Se source may have been difficult to discern.

#### 2.4.5 Calf Plasma and Muscle Se

Selenium concentrations in progeny plasma and muscle are presented in Figures 2.4 and 2.5 and Table 2.7. Calf plasma Se concentrations followed a pattern similar to the cow plasma Se status. Plasma Se in calves did not differ among treatments at 34 d of age ( $P = 0.90$ ) and did not differ for the remainder of the pre-weaning period ( $P \geq 0.11$ ).

All treatment groups reached their lowest plasma Se concentrations at 166 d of age and greatest at weaning which was 216 d of age. This increase in plasma concentrations was likely a result of rapid fall regrowth of forage. At this time all groups had been commingle and were grazing on pasture. This rise was similar to what was also experienced by the cows. Ammerman et al. (1980) reported elevated calf plasma Se as Se supplementation increased in the cow independent of the source, which was attributed to increased Se concentrations in the cow's milk. Gunter et al. (2003) and Guyot et al. (2007) reported a similar increase in calf plasma and whole blood Se as cow Se supplementation increased but also noted that organic Se produced greater increases in plasma Se compared to sodium selenite.

Selenium content of calf muscle biopsies taken at 105 DPP (Figure 2.5) did not differ among treatments ( $P = 0.45$ ). Muscle Se for calves from cows not supplemented with Se, cows supplemented with inorganic Se, and cows supplemented with organic Se were 315, 319, and 360  $\mu\text{g}/\text{kg}$ , respectively. A lack of response in muscle Se due to source and amount may be related to Se status of the basal feeds and previous exposure to Se in the diet. Hintze et al. (2002) observed that steers from a seleniferous background that were supplemented with selenomethionine at 0.62 mg/kg had equal muscle Se concentrations to steers from a nonseleniferous background supplemented with 11.2 mg/kg of selenomethionine. In contrast, sheep supplemented with selenomethionine through high-Se wheat experienced greater muscle Se content, but had lower GPx activity in the muscle compared to sheep supplemented with sodium

selenite, suggesting that the Se from selenite products may be better associated with GPx activity in the muscle compared to organic Se (Van Ryssen et al., 1989). In pigs, liver and muscle Se concentrations were significantly greater when selenomethionine was supplemented compared to sodium selenite (Zhan et al., 2007). Nutritional degenerative myopathy (NDM) is believed to be induced by the lack of Vitamin E and/or Se in the diet. This breakdown of muscle is caused by peroxidative damage in the muscle. Increasing Se concentrations in the muscle will decrease the likelihood of NDM (Walsh et al., 1993).

In conclusion, Se source did not affect cow performance or plasma Se. Cows supplemented with either inorganic or organic Se did have significantly higher liver Se as compared to the control cows. Cows supplemented with organic Se had lower milk protein concentrations than inorganic supplemented cows and elevated milk lactose compared to both inorganic supplemented cows and control cows. Calf performance and tissue Se concentration remained unaffected by maternal Se source. The lack of significant differences between Se treatments could be attributed to previous Se exposure and Se status of cows at trial initiation. None of the cows entered the trial in what would be considered a Se deficient condition. Diets either met or were slightly below recommended NRC (2000) Se intake concentrations.

Table 2.1: Experimental diets fed to cows from 203 D in gestation to 108 DPP

	Gestation	Lactation
Ingredients (% of DM)	Diet	Diet
Corn silage	63.0	39.0
Corn stover	22.4	21.1
Haylage	8.2	7.6
DDGS	6.2	10.1
Soy hulls	-----	22.0
Supplement <sup>1</sup>	0.23	0.23
Nutrient composition		
NEm, Mcal/kg	1.50	1.62
NEg, Mcal/kg	0.90	1.01
Protein, %	10.4	12.1
Calcium, %	0.30	0.35
Phosphorus, %	0.25	0.27
Magnesium, %	0.22	0.22
Vitamin E, IU/kg	440	440
Basal selenium, mg/kg	0.07	0.11
Added selenium, mg/d <sup>2</sup>		
Organic	2.98	2.98
Inorganic	3.05	3.05
Control	0.74	0.74

<sup>1</sup>Supplement contained (DM basis): 13.14% Ca, 0.23% Mg, 0.05% K, 8.6% S, 255.75 mg/kg Co, 3.04% Cu, 0.20% I, 655.46 mg/kg Fe, 6.12% Mn, 0.0% Se, 10.51% Zn, 9369.98 IU/g vitamin A, 937.0 IU/g vitamin D.

<sup>2</sup>Se treatments top dressed daily at 0.454 kg/cow. Topdress contained Selenium (either sodium selenite, Sel-plex, or none), supplement, vitamin E, and salt in DDGS.

Table 2.2: Effect of dietary Se source during late gestation and early lactation on cow BW, ADG, BCS, and DMI

	Treatments			SE	P
	Control	Inorganic	Organic		
Cow weight, kg					
Start	597.5	596.0	593.6	17.42	0.99
Calving <sup>1</sup>	700.6	697.2	691.8	17.42	0.94
Post-calving <sup>1</sup>	636.1	632.1	625.4	17.42	0.90
210 d post-partum	627.0	627.7	609.7	17.42	0.70
Cow ADG, kg/d					
Pre-calving	1.24	1.22	1.18	0.132	0.95
Post-calving	0.24	0.19	0.24	0.132	0.95
Experimental period (0-188 d)	0.34	0.30	0.31	0.132	0.97
Cow BCS					
Start	5.18	5.36	5.23	0.054	0.56
Calving	5.22	5.09	5.13	0.051	0.69
Cow daily DMI, kg/day					
Pre-calving	9.16	8.95	9.23	0.153	0.39
Post-calving	14.4	14.5	14.43	0.153	0.97

<sup>1</sup> Adjusted for gravid uterine weight (Ferrel, 1976). Adjusted weight = pregnant weight -  $743.9e^{(0.02-0.0000143t)t}$

Table 2.3: Effect of dietary Se source during late gestation and early lactation on calving and reproductive performance

	Control	Inorganic	Organic	SE	P
Calving ease <sup>1</sup>	1.27	1.13	1.38	0.178	0.62
Calf vigor	1.07	1.13	1.00	0.064	0.34
Pregnancy rates (%)					
Conception to AI	80.0	66.7	56.3	-----	0.39
Overall	93.3	92.9	93.8	-----	0.99

<sup>1</sup> Calving ease scored on 1-5 score with 1 meaning no assistant, and 5 meaning a caesarian-section. Calf vigor measured on 1-4 scale: 1 meaning nursed on own immediately, and 4 showing that the calf died soon after birth.

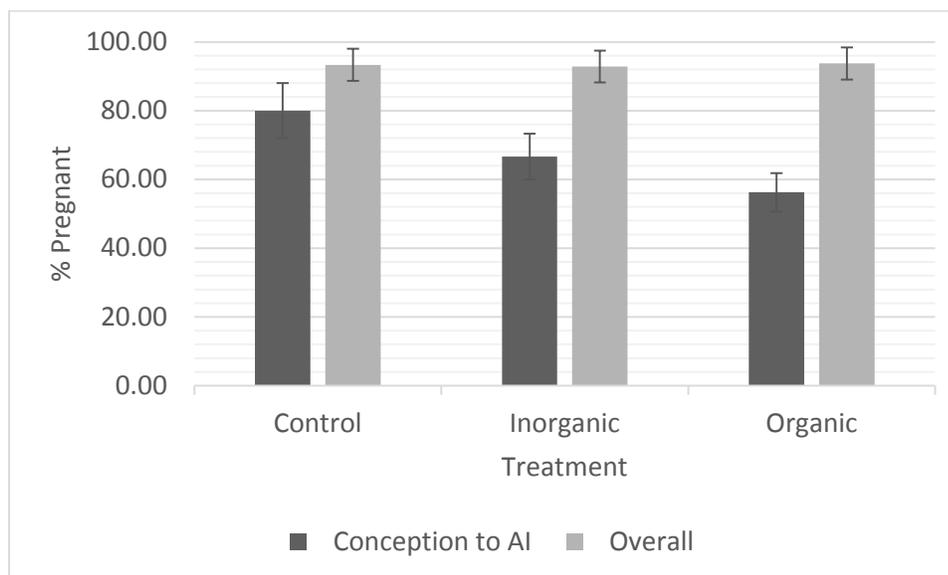


Figure 2.1: Effects of dietary Se source on cow reproductive performance ( $P \geq 0.39$ ).

Table 2.4: Effect of dietary Se source on milk production and composition

	Control	Inorganic	Organic	SE	P
Milk Production <sup>1</sup> , Kg	10.05	9.20	8.68	0.985	0.61
Milk composition <sup>2</sup>					
Fat, %	1.77	1.75	1.43	0.216	0.44
Protein, %	3.19 <sup>ab</sup>	3.33 <sup>a</sup>	3.13 <sup>b</sup>	0.064	0.07
Lactose, %	4.94 <sup>a</sup>	4.96 <sup>a</sup>	5.04 <sup>b</sup>	0.036	0.10
Solids, %	10.92	11.08	10.67	0.212	0.38
Somatic cell count	40.64	51.46	43.88	13.298	0.82
Selenium <sup>3</sup> (µg/l)	22.0	33.0	32.0	5.70	0.32

<sup>1</sup>Milk production measured at 68 DPP using a 12 hour weigh-suckle-weigh.

<sup>2</sup>Milk samples collected at 62 DPP for later composition analysis by Dairy One.

<sup>3</sup>Selenium concentration analyzed using nitric acid digestion.

<sup>ab</sup>Means with different superscripts differ ( $P \leq 0.10$ )

Table 2.5: Effects of dietary Se source on cow plasma and liver Se concentration

	Control	Inorganic	Organic	SE	P
Plasma <sup>1</sup> (µg/kg)					
Day 0	132 <sup>a</sup>	116 <sup>a</sup>	177 <sup>b</sup>	17.0	0.03
Day 111	106	109	123	17.0	0.73
Day 188	85	116	94	17.0	0.37
Day 243	129	108	108	17.0	0.61
Day 267	201	187	184	17.0	0.78
Day 292	177	194	189	17.0	0.77
Liver Se 108 DPP <sup>2</sup> (µg/kg)	287.5 <sup>a</sup>	368.1 <sup>b</sup>	381.0 <sup>b</sup>	22.63	< 0.01

<sup>1</sup>Plasma collected from cows and analyzed for Se concentration. Blood was collected every 28 d from all cows (n=48) through jugular venipuncture.

<sup>2</sup>Liver biopsies were taken at treatment end and Se concentration was analyzed on wet chemistry basis.

<sup>ab</sup>Means with different superscripts differ ( $P \leq 0.01$ )

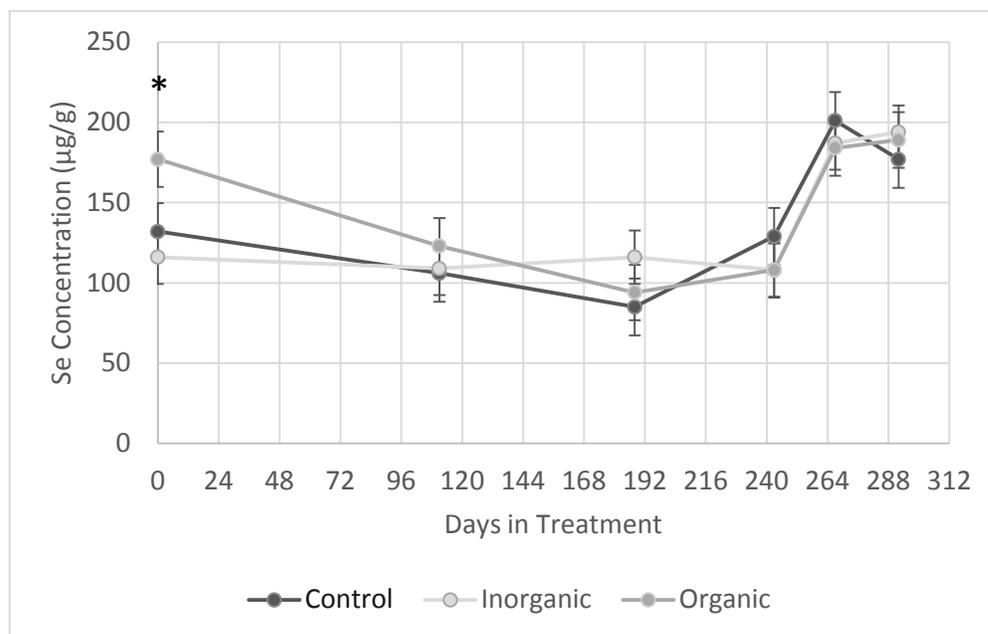


Figure 2.2: Effects of dietary Se source on cow plasma Se concentration. Cows fed organic Se had greater plasma Se concentration of day 0 before beginning of Se

supplementation ( $P = 0.03$ ). No treatment differences observed throughout the remainder of the study ( $P \geq 0.37$ ).

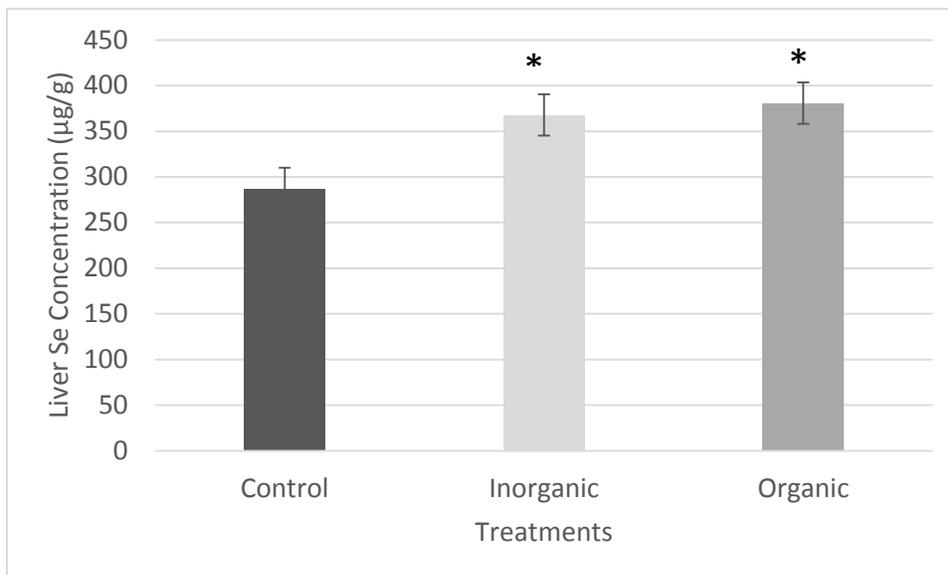


Figure 2.3: Effects of dietary Se source on cow liver Se concentration at 108 DPP. Liver biopsies taken on all cows ( $n=48$ ) at 108 DPP (treatment end) and analyzed on a wet chemistry basis for Se concentration. Cows supplemented with Se had higher liver Se concentration independent of Se source ( $P < 0.01$ ).

Table 2.6: Effects of maternal dietary Se source during late gestation and early lactation on calf weight and ADG

	Control	Inorganic	Organic	SE	P
Calf weight, kg					
Birth	37.1	37.4	38.1	4.62	0.99
108 d post-partum	174.6	174.2	178.5	4.62	0.77
216 d post-partum	284.0	282.6	291.5	4.62	0.33
Calf ADG, kg/d					
Experimental period	1.32	1.32	1.34	0.050	0.94
Post-experimental period	0.99	0.98	1.02	0.052	0.83
Overall	1.15	1.14	1.17	0.052	0.89

Table 2.7: Effects of maternal dietary Se source during late gestation and early lactation on calf plasma and muscle Se concentration

	Control	Inorganic	Organic	SE	P
Plasma ( $\mu\text{g/g}$ )					
Day 34	99	102	110	19.0	0.89
Day 105	103	134	138	19.0	0.30
Day 165	59	71	59	19.0	0.86
Day 190	101	98	111	19.0	0.85
Day 216	195	160	140	19.0	0.11
LM at 108 d DPP ( $\mu\text{g/kg}$ )	315	398	360	5.0	0.45

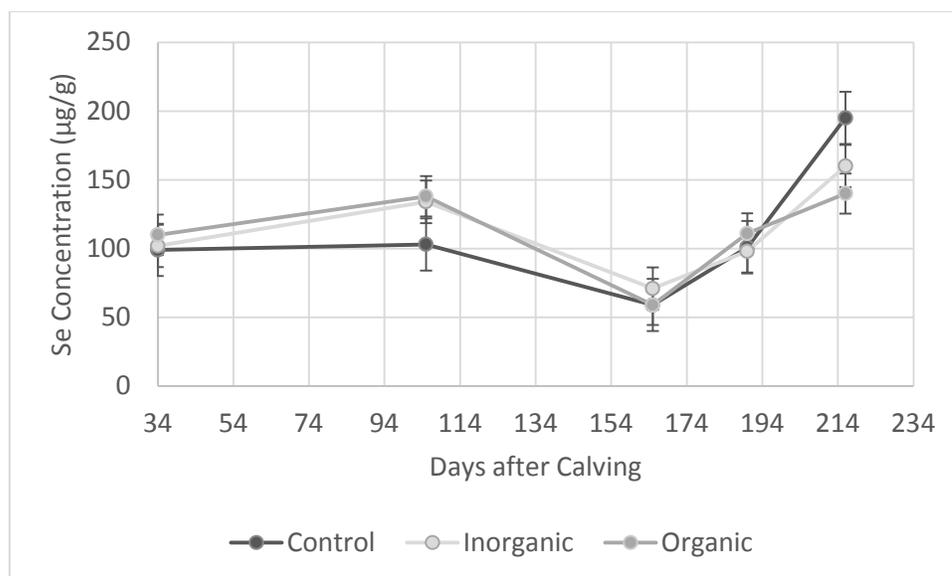


Figure 2.4: Effects of maternal dietary Se source during late gestation and early lactation on calf plasma se concentration ( $P = 0.90$ )

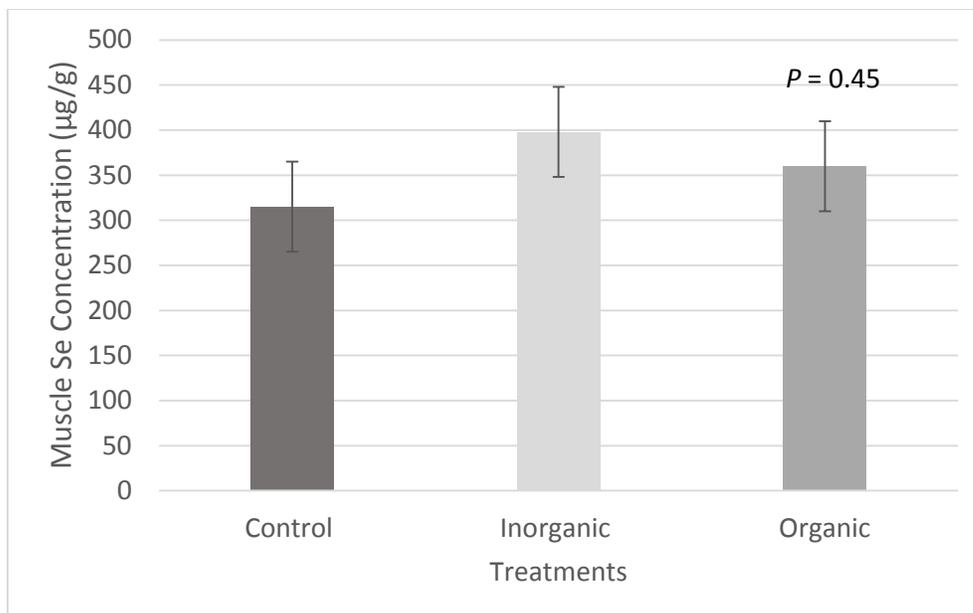


Figure 2.5: Effects of maternal dietary Se source on calf muscle Se concentration at 108 DPP ( $P = 0.45$ )

CHAPTER 3 EFFECTS OF ORGANIC AND INORGANIC SELENIUM SUPPLEMENTATION  
DURING LATE GESTATION AND EARLY LACTATION ON PROGENY FEEDLOT  
PERFORMANCE AND CARCASS CHARACTERISTICS

3.1 ABSTRACT

Angus x Simmental cows (n = 48, BW = 594 kg, BCS = 5.26, Age = 2.7), pregnant with male fetuses, were used to determine the effect selenium (Se) source during the last 80 d of gestation and first 105 d of lactation on progeny feedlot performance. At 203 d in gestation, cows were blocked by body weight, breed composition, and calf sire and randomly allotted to 1 of 3 treatments: no Se, inorganic Se, or organic Se. Maternal diets were formulated to contain 10.4% CP and 0.90 Mcal/kg NEg during gestation and 12.1% CP and 1.01 Mcal/kg NEg during lactation. Basal diets contained 0.07 and 0.11 mg/kg Se for gestation and lactation diets; respectively. Diets were fed daily as a total mixed ration and Se was provided in a top-dress containing 0, 0.30 mg/kg Se as sodium selenite, or 0.30 mg/kg as selenized-yeast (Sel-Plex®). Treatment diets were fed through 105 d post-partum (DPP). At 105 DPP cow-calf pairs were commingled until weaning at 210 DPP. At 28 d post-weaning, steers (n = 47, BW = 301 kg) were placed in individual pens and fed a diet formulated to provide 13.9% CP, 1.24 Mcal/kg NEg, and 0.10 mg/kg Se. The diet was delivered as total mixed ration once daily. Steers from cows

supplemented with organic Se diet entered the feedlot heavier ( $P = 0.02$ ) and tended to be heavier on d 87 ( $P = 0.08$ ) compared to steers from cows supplemented with inorganic Se. There was no difference in ADG among treatments ( $P \geq 0.76$ ), but steers from organic Se cows tended to spend fewer days on feed compared to steers from inorganic Se cows ( $P = 0.09$ ). Steers from organic Se cows had a greater overall DMI compared to steers from inorganic Se cows ( $P = 0.04$ ), but there was no difference in overall gain:feed ratio ( $P = 0.82$ ). Dressing percentage was greater for steers from cows fed no Se compared with steers from cows fed either inorganic or organic Se ( $P = 0.03$ ). Maternal Se source had no effect on hot carcass weight, back fat, %KPH, L. dorsi area, yield grade, marbling score, or quality grade distribution ( $P \geq .17$ ) of progeny. In conclusion, maternal supplementation with organic Se appears to have a long-term benefit on intake of steer progeny and may result in improvements in growth that could decrease days on feed.

### 3.2 INTRODUCTION

Supplementation of Selenium (Se) to cattle is a common practice in mineral programs. The primary source of Se for livestock is from forages and grains, the concentration of which is dependent on the soil Se concentration where the feed was grown. Soil Se concentration varies greatly across the U.S. Areas of the Midwest and Southeast are

marginal to deficient in Se while areas of the Great Plains are adequate to even toxic in Se in localized regions (Dargatz and Ross, 2014).

Adequate Se is vital to an animal's antioxidant defenses. Once ingested, Se is incorporated into the body as a selenoprotein, most commonly selenocysteine. Selenocysteine is a vital component for the production of glutathione peroxidase (GPx), which is produced by the body in order to limit the accumulation of reactive oxygen species (ROS). As an animal is exposed to different stresses, injuries, and toxins throughout its life, there is a buildup of ROS, such as hydrogen peroxide and other free radicals. These free radicals oxidize cell membranes and leads to damage (Miller et al., 1993), which can in turn lead to decreased immune function and fertility, white muscle disease, and poor growth. Glutathione peroxidase works by reducing the harmful peroxides to water, preventing oxidative damage. However, in order for proper GPx production to take place there must be a readily available source of Se. Thus, it is key to maintain adequate Se status in order to achieve animal production goals. Traditionally, sodium selenite has been the common form of Se supplementation. However, sodium selenite is an inorganic sodium salt product that is absorbed poorly in both ruminant and non-ruminant animals. In addition, the natural reducing environment of the rumen further decreases the absorption of selenites (Wright and Bell, 1966). The low absorption of inorganic Se requires a greater amount of supplementation. Selenium found in forages and grains is typically in an organic form, meaning it is chelated to an organic molecule, most typically an amino acid such as cysteine or methionine, and is

absorbed as a Se amino acid, which increases Se bioavailability. In fact, organically bound Se has 120 – 200% the bioavailability of inorganic Se products (Hall et al., 2012; Mahima et al., 2012) and requires lower supplementation. Once consumed, the Se amino acid is incorporated into the body as an amino acid, which increases tissue deposition of Se. Organic Se is produced commercially by growing yeast in Se enriched environment, causing the yeast to produce selenomethionine. Selenized yeast (Se-yeast) increases the Se absorbed in both the tissue and milk, while also increasing the animal's tolerance for greater amounts of Se. Many studies have reported increased blood and tissue Se concentrations (Pehrson et al., 1999; Guyot et al., 2007; Gunter et al., 2014), as well as increased GPx activity (Juniper et al., 2008) when Se-yeast products were used as a Se source. Supplementation of Se through can also increase placental transfer of Se to the offspring (Pehrson et al., 1999).

Maternal supplementation of Se-yeast has been reported to have beneficial effects on the post-weaning performance of the offspring, irrespective of Se supplementation in the offspring's diet. For example, lambs from ewes supplemented with Se-yeast had improved average daily gain and gain:feed compared to lambs from ewes supplemented with inorganic Se (Neville et al., 2010). These results were attributed to decreased oxidative damage and increased vascularity and metabolic activity in the gastrointestinal tract of offspring from feedlot steers supplemented with Se-yeast (Soto-Navarro et al., 2004). Because of the potential for decreased oxidative damage and improved immune function, supplementation with selenized-yeast could

allow cattle to respond better to vaccines, increase dry matter intake, and decrease morbidity, particularly when cattle are transitioned from a milk based diet to a feedlot finishing diet, which can be especially stressful. The majority of disease outbreaks, morbidity, and digestive issues in the feedlot take place within the first 21 days of the animals arrival in the feedlot (Kelly and Janzen, 1986). Ensuring appropriate Se status prior to entering the feedlot could provide a large monetary benefit by cutting death loss, increasing weight gain, and decreasing treatment cost in these animals. In addition, animals supplemented with selenized-yeast could produce Se-enriched meat for consumers. For example, Juniper et al. (2008) reported a 48% increase in Longissimus Muscle (LM) Se retention when organic Se-yeast was supplemented compared to inorganic sodium selenite. Lambs supplemented with Se-yeast had 56% increase in the LM compared to lambs fed the same concentration of sodium selenite (Vignola et al., 2009).

Although a large amount of work has been performed demonstrating the benefits of supplementing both maternal and growing ruminants with selenized-yeast compared to inorganic compounds, little focus has been placed on examining the effects of maternal supplementation of Se on post-weaning performance of the offspring. Thus, our hypothesis was that inclusion of organic selenium in gestating and lactating beef cow diets would improve selenium status and long-term growth of male progeny compared with inclusion of inorganic Se. Our objective was to quantify the

effects of maternal organic and inorganic selenium on feedlot performance and selenium status in male progeny associated with developmental programming.

### 3.3 MATERIALS AND METHODS

The study was performed at the Purdue Animal Sciences Research and Education Center in West Lafayette, IN. All procedures and protocols followed guidelines in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010) and were approved by the Purdue Animal Care and Use Committee.

Angus x Simmental cows (n = 48), confirmed pregnant to male fetuses, were used to determine the effects of selenium (Se) source and supplementation during the last 80 d of gestation and first 105 d of lactation on feedlot performance of male progeny. At 203 d of gestation, cows were blocked by BW, breed composition, age, and calf sire and then randomly allotted to one of three treatments, which consisted of no supplemental Se, 3 mg/d inorganic Se (sodium selenite), and 3 mg/d organic Se as selenized-yeast (Sel-Plex®, Alltech Inc., Nicholasville, KY). Cow diets during gestation were isocaloric (0.90 Mcal/kg NEg) and isonitrogenous (10.4% CP) and cow diets during lactation were isocaloric (1.01 Mcal/kg NEg) and isonitrogenous (12.1% CP). Basal diets provided 0.07 mg/kg and 0.11 mg/kg of Se for the gestation and lactation diets. Treatment diets were fed to cows until 105 DPP at which point cows and calves were commingled and turned out to pasture for the remainder of the summer. One steer was removed from the study for reasons unrelated to treatment. Steers were weaned at 216 d of age and placed on pasture for 28 d and adapted to a common feedlot diet (Table

2.1). At 244 d, 47 steers were placed into individual feeding pens (2.7 x 1.5 m) on slatted floors, in a curtain-sided finishing barn.

At feedlot entry calves were vaccinated against bovine rhinotracheitis, bovine viral diarrhea, parainfluenza-3, bovine respiratory syncytial virus, (Bovi-Shield Gold FP5<sup>®</sup>; Zoetis, Florham Park, NJ), *Haemophilus somnus*, *Pasturella*, and *Clostridia* (Vision-7 Somnus; Merck Animal Health, Summit, NJ), and treated with an anthelmintic (Valbazen<sup>®</sup>, Zoetis) for internal and external parasites, and implanted with Revalor-XS<sup>®</sup> (4 mg estradiol and 20 mg trenbolone acetate; provided courtesy of Merck Animal Health).

### 3.3.1 Diets

Steers were fed a common diet consisting of 40.0% corn, 20.0% corn silage, 35.0% dried corn gluten pellets, and 5.0% mineral until slaughter (Table 2.1). There was no added Se in the mineral, however the basal diet contained 0.10 mg/kg of Se. The diet was formulated to meet or exceed NRC (2000) requirements for protein (13.9%), energy (1.24 Mcal/kg), vitamins, and minerals except for Se, for a finishing beef steer (NRC, 2000). Diets were fed ad libitum using the South Dakota State 4-point bunk scoring system (Pritchard, 1993) to achieve a score of 0.5 for morning bunk checks. Water was offered free choice and the diet was delivered as a TMR once daily at 0800 h. Individual feed intakes were recorded daily and refusals were weighed and discarded. Bi-weekly feed samples were taken and dry matter content was recorded after drying samples in a 60°C force air oven for 72 h. Feed ingredient subsamples were composited and analyzed

for chemical composition by wet chemistry methods (AOAC, 1990; Sure-Tech Laboratories, Indianapolis, IN).

### 3.3.2 Body Weights and Blood Collection

Initial and final steer BW were determined by averaging two consecutive day weights. Individual BW's were taken monthly to determine feedlot performance. After harvest, dressing percentages were calculated by dividing the hot carcass weight by the live weight of each steer and multiplying by 100. Carcass adjusted final live weights were also calculated for individual animals using the average dressing percentage for all treatments (61.57%). Carcass adjusted performance was calculated using the carcass adjusted final weight.

Pre-prandial blood samples were collected monthly via jugular venipuncture using 10 ml Vacutainer tubes containing 158 USP Sodium Heparin (Becton Drive, Franklin Lake, NJ). Blood was inverted and placed on ice until centrifugation at 500 x g for 20 minutes at 4°C. Plasma was separated and stored at -20°C freezer until analysis for Se. Steers were slaughtered when a target BW of approximately 590 kg was achieved. Twenty-Eight steers were slaughtered at a commercial abattoir (Tyson, Joslin, IL) and the additional 19 steers (6 or 7 steers per treatment) were slaughtered at the Purdue University meat lab in West Lafayette, IN. Hot carcass weight was recorded immediately after evisceration. After a 24-h chill, 12<sup>th</sup> rib fat thickness, longissimus (LM) area, kidney pelvic heart fat (KPH), yield grades, marbling scores and quality grades were determined by trained University personnel.

### 3.3.3 Muscle and Liver Sampling

Longissimus muscle biopsies were taken from steers 10 d prior to harvest according to the procedures of Pampusch et al. (2008). Steers were restrained in a hydraulic squeeze chute, hair was removed from the biopsy site and a local anesthetic (lidocaine HCl; 20 mg/mL; 8 mL per biopsy) was administered. Biopsies were obtained from between the 10<sup>th</sup> and 13<sup>th</sup> rib from alternate sides for sequential samples. The biopsy site was cleansed with Betadine for the initial scrub followed by alcohol. A 1-cm incision was made with a scalpel and a sterile Bergstrom biopsy needle (Stille Surgical, Lombard, IL) was used to obtain 1 g of tissue from the longissimus muscle. The incision was rinsed with sterile saline, a topical antibiotic spray was applied and the incision site was covered with a spray-on aluminum bandage. Samples were flash frozen with liquid nitrogen then placed in a -80°C freezer for later analysis of Se content.

Approximately 1 g of liver tissue was collected immediately after slaughter from the subsample of 19 steers slaughtered at the Purdue University meat laboratory. Steers were selected based on the average body weight of each treatment: 6 from inorganic Se, 6 organic Se, and 7 from no Se supplementation. Liver samples were flash frozen with liquid nitrogen and placed in a -80°C freezer until analysis of Se.

### 3.3.4 Selenium analysis

Muscle (0.5 g), and plasma (3.0 mL) samples were analyzed for Se content according to AOAC procedure 996.16 (AOAC, year). Samples were digested in duplicate

in nitric and perchloric acid (4:1, v:v). Samples were then derivatized using EDTA and diaminonaphthalene solutions, extracted with cyclohexane, transferred to a white well plate (Dyner Technologies Inc., Chantilly, VA) and analyzed for Se in a fluorometer (Magellan GENios Pro, Tecan Trading Ag, Switzerland) using an excitation wavelength of 375 nm and emission wavelength of 525 nm. Liver samples were shipped to Alltech Inc. in Nicholasville, KY for Se analysis (K.M. Brennan et al., 2011)

### 3.3.5 Statistical Analysis

Steer BW, ADG, DMI, and plasma Se were analyzed using the MIXED procedure of SAS for repeated measures (Version 8.0, SAS Inst. Inc., Cary, NC). The covariance structures autoregressive order one, heterogeneous autoregressive order one, unstructured and compound symmetric were compared and the covariance structure with the smallest Bayesian information criterion was chosen for analysis results. Carcass characteristics and Se content of muscle and liver samples were analyzed using MIXED procedure of SAS as a completely randomized design. Carcass quality distribution was measured using the GLIMMIX procedure of SAS. Animal was the experimental unit and the model included the fixed effects of treatment and day, along with appropriate treatment x day interactions. The SLICE function of SAS was used to determine simple effects within day. For all variables analyzed, a  $P$ -value  $\leq 0.05$  was identified as significant, while  $0.05 > P \geq 0.10$  was identified as a tendency approaching significance.

### 3.4 RESULTS AND DISCUSSION

#### 3.4.1 Performance

Performance data is presented in Table 3.2. Steers from cows supplemented with organic Se tended to be heavier ( $P = 0.06$ ) at feedlot entry compared to steers from cows supplemented with inorganic Se; steers from cows not supplemented with Se did not differ in feedlot entry weight compared to steers from cows fed either Se source (301, 292, 308 kg for control, inorganic, and organic Se progeny, respectively). There was no treatment effect for BW at the midpoint of the feedlot phase or at slaughter ( $P = 0.63$ ), nor was there a difference ( $P = 0.61$ ) among treatments for carcass-adjusted BW at slaughter (623, 630, and 637 kg, respectively for control, inorganic, and organic progeny). There was no effect of maternal treatment on steer progeny average daily gain (ADG) for the first-half of the feedlot phase ( $P = 0.97$ ), second-half of the feedlot phase ( $P = 0.42$ ), or overall ( $P \geq 0.42$ ). Although overall average daily gains did not differ (1.75, 1.76, and 1.86 kg/d, respectively for control, inorganic and organic treatments), steers from cows supplemented with organic Se tended to spend fewer days in the feedlot compared to steers from cows supplemented with inorganic Se. Neither Se treatment differed from control for days on feed (188, 191, 176 d for control, inorganic, and organic, respectively)., Steers from organic Se supplemented cows consumed 1.5 kg/d more than steers from the inorganic Se supplemented cows during the first-half of the feedlot phase ( $P = 0.02$ ), 0.9 kg/d more during the second-half of the feedlot phase ( $P = 0.05$ ) and 0.9 kg/d more overall ( $P \leq 0.04$ ). Dry matter intake for steers from control

cows did not differ during any phase compared to steers from cows supplemented with organic or inorganic Se ( $P \geq 0.50$ ). Improving DMI for steers entering the feedlot could improve immune response and help to prevent respiratory disease. The majority of disease outbreaks, morbidity, and digestive issues in the feedlot take place within the first 21 days of the animals arrival in the feedlot (Kelly and Janzen, 1986). Ensuring that these animals have appropriate Se status prior to entering the feedlot could provide a large monetary benefit to producers by cutting death loss, weight loss, and treatment cost in these animals.

In the current study, the elevated DMI of the steers from organic supplemented cows and no improvement in ADG tended to result in poorer a gain:feed ratio during the first-half of the feedlot period compared to steers from cows supplemented with inorganic Se ( $P = 0.06$ ). Gain:feed for steers from cows not supplemented with Se did not differ from either maternal Se source treatment during the first 80 d of the study. During the second-half of the feedlot phase and overall, there was no maternal treatment effect on feedlot steer progeny gain:feed ( $P \geq 0.49$ ). In broilers, organic Se increased DMI and ADG compared to inorganic Se (Sevcikova et al., 2006). In addition, maternal supplementation of organic Se improved pre-weaning calf ADG compared to inorganic Se (Guyot et al., 2007) and maternal supplementation of supranutritional selenomethionine to overfed ewes improved ADG and gain:feed in lamb progeny compared to overfed ewes that were only fed adequate selenomethionine (Neville et al., 2010). In contrast, Lawler et al. (2004) reported no difference in ADG when steers

were fed supranutritional concentrations of Se with either sodium selenite or high-Se wheat (selenomethionine) and did not observe an effect on gain:feed. In guinea pigs, supranutritional Se actually decreased ADG compared to adequate Se (Chaudhary et al., 2010). This suggests that the benefits of organic selenium supplementation is dependent on several other factors in the maternal diet, including previous exposure to Se, basal Se concentrations in the diet, and the amount of feed being delivered to the animal.

#### 3.4.2 Carcass Characteristics

Carcass characteristics are presented in Table 3.3 and Figure 3.1. There was no effect ( $P = 0.29$ ) of maternal Se treatment on hot carcass weight (393, 388, and 384 kg, respectively; for the control, inorganic, and organic treatments). Steers from cows not supplemented with Se had a greater dressing percentage ( $P = 0.03$ ) compared to steers from cows supplemented with inorganic or organic Se, (62.5, 61.6, and 61.1%, respectively for control, inorganic, and organic treatments, respectively). No treatment effects were observed among groups for fat thickness, ribeye area, kidney pelvic heart percentage, yield grade or marbling score ( $P \geq 0.17$ ). No other previous studies have reported an effect of source or amount of Se on dressing percentage in feedlot steers (Hintze et al., 2002; Lawler et al., 2004) or chickens (Sevcikova et al., 2006). It is possible in the present study that decreased DMI in control steers may have decreased visceral organ mass resulting in a higher dressing percentage than that of steers from cows supplemented in inorganic and organic Se. Fluharty et al. (1999) reported that even with

similar energy and protein intake, lambs that had decreased DMI with similar or elevated ADG had lower visceral organ mass. This in turn could decrease dressing percentage (Fluharty et al., 1999).

#### 3.4.3 Plasma, Muscle, and Liver Se Concentrations

Plasma, muscle, and liver Se concentrations are presented in Table 3.4 and Figures 3.2 and 3.3. No treatment effects were observed at any time point among maternal Se treatments ( $P \geq 0.61$ ) for feedlot steer progeny plasma Se concentration. All treatments experienced the greatest plasma Se concentration at feedlot entry (166, 185, and 172  $\mu\text{g}/\text{kg}$  for the control, inorganic and organic treatments, respectively) and plasma Se subsequently decreased throughout the feedlot period until day 112 at which point all groups reached their lowest plasma Se concentration ranging from 112 to 115  $\mu\text{g}/\text{kg}$ . Plasma Se concentrations for the control, inorganic, and organic treatments did rise slightly by the last collection time-point, to 116, 126, and 137  $\mu\text{g}/\text{kg}$ , respectively. No differences ( $P = 0.52$ ) among treatments for muscle Se concentration were noted (414, 388, and 562  $\mu\text{g}/\text{kg}$ , respectively for control, inorganic, and organic treatments). Liver Se concentration did not differ ( $P = 0.63$ ) between steers from cows fed the control, inorganic, or organic Se diets (353, 307, and 374  $\mu\text{g}/\text{kg}$ , respectively). Similar to the present study, plasma and liver Se concentration in steers fed corn-based or molasses-based diets supplemented with sodium selenite or organic Se did not differ (Arthington, 2008), potentially because of a high sulfur content in the molasses and the antagonistic effect on sulfur has on Se absorption. For example, when sulfur

concentrations were increased by 30% in a dairy cow diet, Se digestibility was decreased by 42.3% (Ivancic and Weiss, 2001). The diet fed to steers in the current study included corn gluten feed at 35.0% of the diet DM, which contributed to a sulfur concentration in the total diet of 0.26 %. Although 0.26% sulfur is considered to be below a toxicity threshold, the dietary sulfur may still have decreased the availability of Se to the steers in all treatments, particularly because Se was not added to the feedlot diet.

In contrast to the present study, heifers supplemented with organic Se and a mix of inorganic and organic Se had elevated serum, and liver Se concentrations as compared to heifers supplemented with inorganic Se (Kristen M Brennan et al., 2011). Similarly, steers fed supranutritional concentrations of selenomethionine, had significantly higher concentrations of Se in the muscle and liver compared to steers fed supranutritional concentrations of sodium selenite (Lawler et al., 2004). Although, in these studies Se was directly supplemented to the animals rather than through maternal transfer. However, increased maternal Se transfer to the steers in the present study was expected to deliver similar results. According to Gunter et al. (2003), maternal supplementation of organic Se to cows elevated the Se status of the calves at birth compared to calves from cows supplemented with inorganic Se. For the current study, the basal Se concentration of the diet for all steers was 0.10 mg/kg despite no added Se, which meets the recommended values for daily Se intake (NRC, 1996). The majority of reports indicating increased Se absorption due to Se source was fed to cows whose diets were deficient in Se. Hintze et al. (2002) reported that animals may physiologically adapt

nutrient absorptive capacity based on the availability of the nutrient, thus Se deficient animals may become more efficient in their absorption of Se. Once fed a Se-adequate diet, absorption of Se by previously Se-deficient cows, particularly an organic source of Se, may be greatly enhanced.

In conclusion, maternal supplementation with organic Se appears to have a long-term benefit on intake of steer progeny and may result in improvements in growth that decreases days in the feedlot.

Table 3.1: Feedlot Diets

Ingredient (% DM)	diet
Corn %	40.0
Corn silage %	20.0
Gluten feed %	35.0
Supplement <sup>1</sup> %	5.0
NE <sub>m</sub> , Mcal/kg	2.11
NE <sub>g</sub> , Mcal/kg	1.24
Protein, %	13.90
Calcium, %	0.86
Phosphorus, %	0.63
Potassium, %	1.08
Selenium, mg/kg	0.10
Sulfur, %	0.26

<sup>1</sup>Vitamin/mineral pre-mix contained (DM basis): 15.36% Ca, 0.39% Mg, 0.92% K, 0.17% S, 3.55 mg/kg Co, 160.56 mg/kg Cu, 8.96 mg/kg I, 449.18 mg/kg Fe, 406 mg/kg Mn, 6.32 mg/kg Se, 501.16 mg/kg Zn, 38.4 IU/g vitamin A, 4.55 IU/g vitamin D, 134 IU/kg vitamin E, 386.5 mg/kg Rumensin (176.4 g/kg, Elanco Animal Health, Indianapolis, IN), 119.3 mg/kg Tylan (88.2 g/kg, Elanco Animal Health, Indianapolis, IN)

Table 3.2: Effects of maternal dietary Se source during late gestation and early lactation on progeny feedlot performance

	Control	Inorganic	Organic	SE	P
<b>Weight</b>					
Feedlot entry	301.0 <sup>ab</sup>	292.2 <sup>a</sup>	308.3 <sup>b</sup>	7.70	0.06
Midpoint	463.3	457.0	473.7	7.70	0.14
Harvest	622.9	622.2	628.8	7.66	0.78
Adjusted Harvest <sup>1</sup>	637.4	629.8	623.2	10.3	0.61
<b>ADG (kg/day)</b>					
First half	1.87	1.89	1.90	0.100	0.97
Second half	1.67	1.68	1.83	0.100	0.42
Overall	1.75	1.76	1.85	0.100	0.73
Adjusted overall <sup>1</sup>	1.82	1.79	1.81	0.254	0.97
<b>DMI (kg/day)</b>					
First half	9.4 <sup>ab</sup>	8.5 <sup>a</sup>	10.0 <sup>b</sup>	0.37	0.02
Second half	12.5 <sup>ab</sup>	12.2 <sup>a</sup>	13.1 <sup>b</sup>	0.30	0.05
Overall	10.9 <sup>ab</sup>	10.5 <sup>a</sup>	11.4 <sup>b</sup>	0.25	0.04
<b>Gain:feed</b>					
First half	0.202 <sup>ab</sup>	0.222 <sup>a</sup>	0.194 <sup>b</sup>	0.0090	0.06
Second half	0.141	0.139	0.131	0.0090	0.62
Overall	0.166	0.172	0.161	0.0090	0.49
Adjusted overall <sup>1</sup>	0.167	0.170	0.159	0.0090	0.49
Days on feed	187.6 <sup>ab</sup>	191.3 <sup>a</sup>	175.5 <sup>b</sup>	5.41	0.09

<sup>1</sup>Adjusted with a common average dressing percent (61.57%) from the trial across all treatments.

<sup>ab</sup>Means with different superscripts differ ( $P \leq 0.10$ )

Table 3.3: Effects of maternal dietary Se source during late gestation and early lactation on progeny carcass quality

	Control	Inorganic	Organic	SE	P
Hot carcass weight (kg)	392.5	387.9	383.7	4.0	0.29
Dressing, %	62.5 <sup>a</sup>	61.6 <sup>b</sup>	61.1 <sup>b</sup>	0.53	0.03
Fat thickness, cm	1.55	1.27	1.30	0.109	0.17
L. dorsi area, cm <sup>2</sup>	88.26	87.04	85.36	1.594	0.27
Kidney, pelvic, heart fat, %	2.0	2.1	2.1	0.06	0.51
Yield grade	3.29	3.11	3.17	0.16	0.72
Marbling score	382.1	384.5	343.1	19.86	0.25

<sup>ab</sup>Means with different superscripts differ ( $P \leq 0.05$ ).

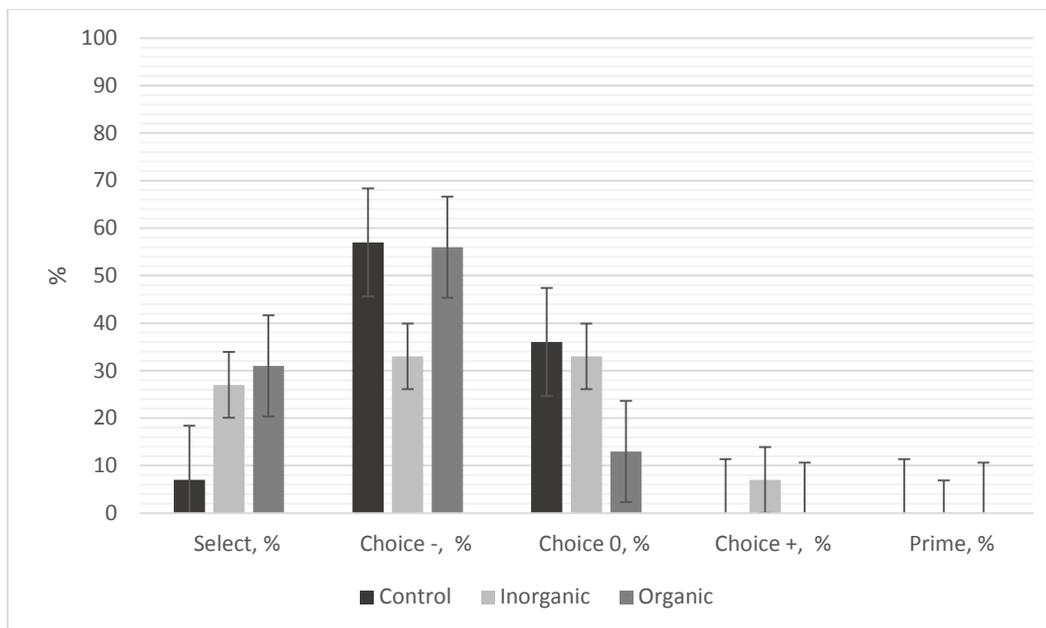


Figure 3.1: Effects of maternal dietary Se source during late gestation and early lactation on progeny quality grade distribution ( $P \geq 0.31$ )

Table 3.4: Effects of maternal dietary Se source during late gestation and early lactation on progeny plasma, liver, and muscle Se concentrations ( $\mu\text{g/g}$ )<sup>1</sup>

	Control	Inorganic	Organic	SE	P
Plasma					
Day 0	166	185	172	10.6	0.63
Day 28	145	163	155	10.6	0.70
Day 55	144	139	145	10.6	0.88
Day 87	128	114	133	10.6	0.52
Day 112	115	112	112	10.6	0.98
Day 135	126	132	127	10.6	0.97
Day 168	116	126	137	10.6	0.61
Liver Se at slaughter	353	307	374	49.5	0.63
Muscle Se at slaughter	414	388	562	122.0	0.52

<sup>1</sup>Blood was collected every 28 d from steers ( $n=47$ ) and plasma was analyzed for Se concentration. Liver biopsies were taken immediately after harvest from subset of steers ( $n=19$ ) and analyzed on wet chemistry basis for Se concentration. Muscle biopsies were taken 10 d prior to harvest and analyzed for Se concentration on wet chemistry basis.

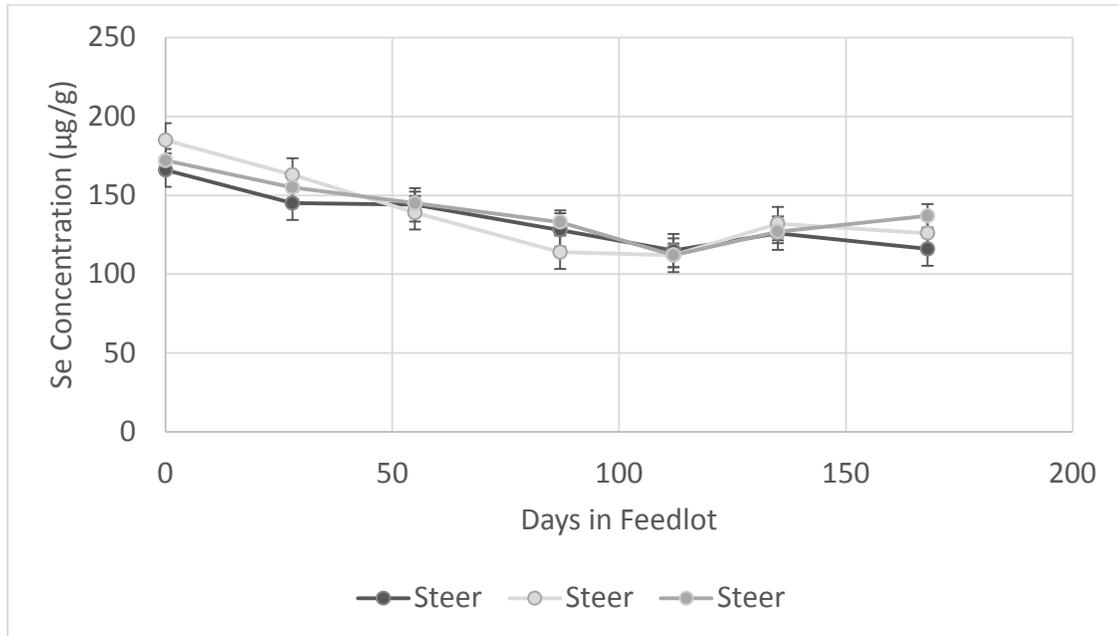


Figure 3.2: Effects of maternal dietary Se source during late gestation and early lactation on progeny plasma Se concentration ( $P \geq 0.52$ ).

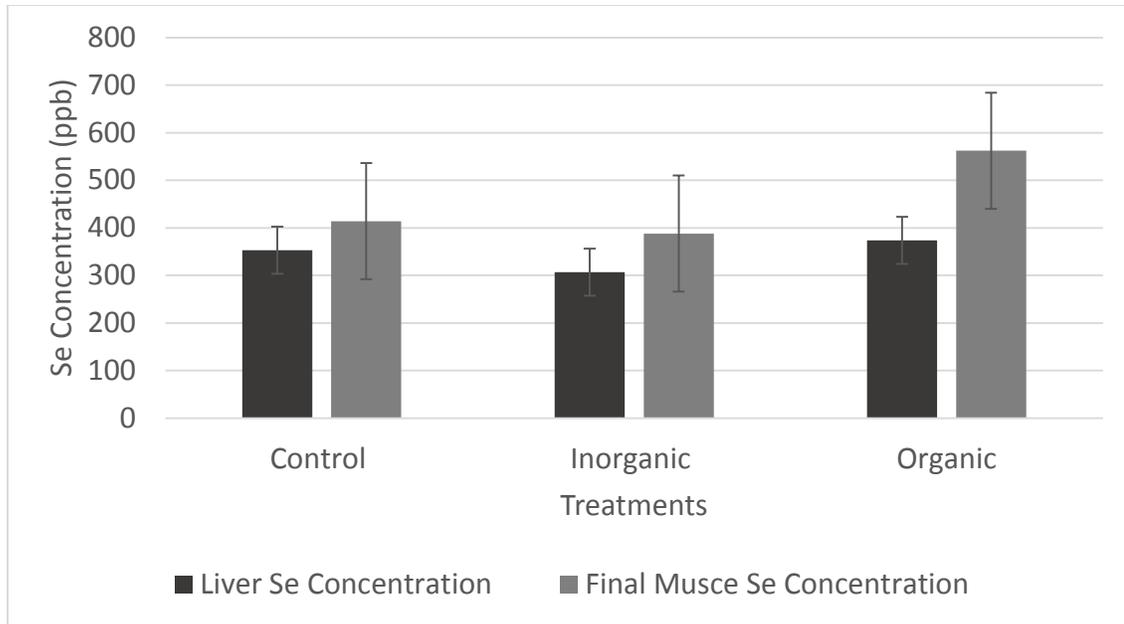


Figure 3.3: Effects of maternal dietary Se source during late gestation and early lactation on progeny liver and muscle Se concentration at harvest ( $P > 0.51$ ).

## CHAPTER 4 CONCLUSIONS AND FUTURE DIRECTION

Mineral supplementation to gestating and lactating ruminants is key to both the health of the dam and her offspring. A proper balance in mineral status ensures the opportunity for proper growth, immune function, and future performance. When animals become deficient or toxic in the mineral concentrations, both the dam and the offspring will suffer. However, different mineral sources and forms can affect absorption and therefore impact the amount of mineral that must be provided in the diet. Work has shown that certain organic or chelated minerals have increased absorption, retention, and passive capabilities as compared to their inorganic counterparts. Selenium has traditionally been supplemented as the inorganic salt; sodium selenate. Selenomethionine is the common form of Se naturally found in forages and grains, and is now commercially produced by growing yeast in a Se enriched environment to produce Se-yeast. Research has shown an increased maternal transfer of organic Se through milk which has resulted in improved Se status in the offspring when supplemented during gestation and/or lactation. Very little work has followed the progeny past weaning and into maturity. Our hypothesis was that supplementing organic Se to cows during late gestation and early lactation would improve the

performance of the cow and her progeny compared to supplementing cows with inorganic Se.

In chapter 2, we demonstrated that there was no improvement in performance or Se status in cows supplemented with organic Se. As expected, liver Se concentrations did improve with Se supplementation but did not differ between inorganic and organic minerals. We saw no increased Se concentration in milk. Although Se was not supplemented to the cows in the control group, the basal concentration of Se in the diet was only slightly below what is recommended by the NRC. This may have limited effects to cows supplemented with either organic or inorganic Se. Also, vitamin E was included in the diets, which works with Se in antioxidant defense. Many of the studies demonstrating positive effects due to organic Se supplementation, examine cows deficient in Se. Selenomethionine may have also been indiscriminately incorporated as methionine, tying the Se up in tissues. This would then limit the capabilities of increasing the Se status through selenomethionine products. However, it has been shown that in time of deficient mineral status, animals have the ability to up-regulate the absorption capacity of minerals. This may suggest that these animals would have used the Se in selenomethionine to meet needs if they were under deficient circumstances.

These results were similar to what we examined in the offspring of each treatment groups. Birth weight, calf vigor, and calving ease remained unaffected by maternal supplementation. Calf weights and ADG also remained unaffected by maternal supplementation throughout the pre-weaning period.

This lack of difference in calving weights and growth may be due to Se concentrations of the cow's milk being unaffected by organic Se supplementation. None of the cows ever reached a plasma Se concentration that would suggest a deficiency that would have resulted in nutritional myodegeneration or white muscle disease. Selenium concentrations remained at a concentration that assured proper antioxidant function and defense. This allowed for appropriate muscle growth and immune function in the calves. If significant differences would have been observed between cows in Se concentrations, I would have hypothesized that we would have seen a difference in calf performance. Although, there was a difference in liver Se concentrations between both supplemented groups and the control group of cows, all concentrations remained in the tolerance concentration of ruminants, limiting any positive conclusions that could have been made through the passage of Se from the cows to their calves.

In Chapter 3, we observed that calves tended to enter the feedlot at a heavier weight when supplemented with organic Se as compared to inorganic Se. This could have been due to an increase in their immune response during the stressful time of weaning, however there were no difference in plasma Se concentrations. A difference in weight was not seen at any point of the feedlot trial. Steers from cows supplemented with organic Se did have a significant increase in DMI through the feedlot trial as compared to steers from cows supplemented with inorganic Se. It is possible that the organic selenomethionine mineral was absorbed as methionine, resulting in increased muscle maintenance which may have required a higher energy intake from the diet. Although ADG did not differ, the increased DMI coupled with a minor

advantage for weight gain likely led to a decreased days on feed for steers from cows supplemented with organic Se.

In conclusion, organic Se supplementation to cows during late gestation and early lactation did not affect the Se status of cows or their progeny compared to inorganic Se. This trend remained true once the offspring entered into the feedlot and through slaughter. None of the diets fed in this study were severely deficient in Se concentrations and all included adequate vitamin E. Future research should examine gestating and lactating diets that are deficient to marginal in both Se and vitamin E. This should also remain true in the feedlot rations provided to the offspring. One key aspect of future research should focus on the increased DMI and decreased days on feed that was observed in steers from organic supplemented cows as compared to steers from inorganic supplemented cows in the current study. Maintaining proper DMI is crucial to the health of incoming calves and their feedlot performance. A numerical increase in ADG was also observed in these steers which led to the decreased DOF for steers from organic supplemented cows. Maintaining a healthy DMI in feedlot steers will promote proper immune function and growth. Coupled with decreased DOF this can result in decreased financial inputs for producers. These changes in DMI and ADG also affect DP which is crucial to the carcass quality of animals produced. Organ weights and visceral mass measurements could be examined after slaughter to expose any effects.

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VITA

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Christopher R. Muegge

Graduate School, Purdue University

Education

B.S., Animal Science, 2011 Oklahoma State University, Stillwater, Oklahoma

M.S., Animal Science, 2015 Purdue University, West Lafayette, IN

Research Experience

Research Assistant August 2013 – Present

- Communicated and coordinated studies with both University farm managers and professors
- Managed studies involving both gestating cows and feedlot steers
- Extracted Se from plasma, milk, and tissue samples
- Performed multiple total collections on feedlot steers
- Managed commercial study at offsite, private feedlot
- Maintained inventory of lab supplies
- Responsible for statistical analysis using SAS

Certifications

Purdue Animal Care and Use Committee (PACUC) Qualified

Radiological and Environmental Management (REM) Certified

Animal Experience

Alltech, Nicholasville, Ky. Research Student 2013-2015

- Performed total collections and glucose tolerance tests at Purdue University

Ault Farms, Rochester, IN Consultant 2015

- Performed on-farm trial examining different varieties of silage
- Implemented new bunk and feed management strategy

#### CGS Feedlot, Cattle Manager 2011-Present

- Managed 2,000 head feedlot and 300 head cow herd
- Responsible for cattle procurement and marketing
- Managed health program, vaccination protocols, and quality control
- Managed feed purchase and ration formulation
- Responsible for 6 other employees

#### Abstracts

Muegge, C. R., K. M. Brennan, R. P. Lemenager, and J. P. Schoonmaker. 2014. Effect of inorganic or organic selenium supplementation during gestation and lactation on cow and pre-weaning calf performance. *J. Anim. Sci.* 92(Suppl. 1):345.

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Muegge. C. R., R. P. Lemenager, and J. P. Schoonmaker. 2015. Effect of the addition of calcium oxide in soybean hull and non-soybean hull based beef diets on feedlot performance and carcass characteristics. Presented at the 2015 American Society of Animal Science Meetings.