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INFLUENCE OF DIETARY SELENIUM SUPPLEMENTATION FORM ON
HEPATIC TRANSCRIPTOME PROFILES OF MATURING BEEF HEIFERS

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture
at the University of Kentucky

By

Zhi Zhang

Lexington, Kentucky

Director: Dr. James C. Matthews, Professor of Animal and Food Sciences

Lexington, Kentucky

2012

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ABSTRACT OF THESIS

INFLUENCE OF DIETARY SELENIUM SUPPLEMENTATION FORM ON HEPATIC TRANSCRIPTOME PROFILES OF MATURING BEEF HEIFERS

Our objective was to know how the hepatic transcriptome expression of growing beef (Angus-cross) heifers (0.5 kg gain/day) was affected by the feeding of different sources of dietary (3 mg/day) Se supplements: inorganic Se (ISe, sodium selenite), organic (OSe, Sel-Plex®), or a blend (1.5 mg:1.5 mg) of ISe:OSe (Mix), compared to the adequate but non-Se supplemented “Control”. The biopsied hepatic tissues of these four groups heifers collected at day 168 (when liver Se assimilation had stabilized) after supplements of Se, was subjected to the microarray analysis to assess Se treatment effects.

The results suggest that there were clear differences in the hepatic gene expression profile of the four Se treatment groups. 139 significantly treatment-induced differentially expressed transcripts were selected. Among them: 1) the gene expression profiles of Control and OSe appeared to be more similar than Control and ISe, 2) eight distinct gene expression patterns among treatments were identified and each of them indicates affected biofunctions and networks, 3) they were grouped as the expression profile relative to Control, there were solely and commonly affected transcripts for four Se treatments and they indicated different biofunctions, 4) of them, three microRNAs were identified and their predicated mRNA targets showed different biofunctions.

KEYWORDS: Bovine, Liver, Microarray, Biofunctions, MicroRNA

Zhi Zhang

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Chapter 1: Introduction

As the largest segment of the American agricultural economy, the cattle industry comprises approximately 1.23 million businesses with cattle, including beef and dairy cattle. An estimated 1.064 million farmers and ranchers raise beef cattle in the United States (Kentucky Beef Book, 1997). As one of the largest beef producing states east of the Mississippi river, Kentucky is home to over 1.16 million beef cows with a total cattle inventory of some 2.40 million head (Kentucky Beef Council, 2010). Cattle producers generate more than \$605 million dollars in cash sale receipts in 2007 (Kentucky Beef Council, 2010) According to recent data, beef cattle are produced on 45,000 (50.6 percent) of Kentucky's 89,000 farms (Kentucky Beef Book, 1997). Therefore, cattle production is very important to many farmers in Kentucky.

Se is a metalloid belonging to Group VIA of the Periodic Table, the same as sulfur. Selenium plays an important role in regulation of various physiological functions in beef cattle including immunity, reproduction and early postnatal viability (Suzuki, 2005). Se deficiency alone, or in combination with vitamin E, is associated with reduced growth and productivity rates (Surai, 2006), early mortality (Surai, 2006) and immune-suppression (Surai, 2006). Se deficiency has been specifically linked with the development of various diseases in ruminants (Pehrson, 1993; Kolb and Seehawer, 2001) including nutritional muscular dystrophy (white muscle disease, the most well documented disease related to Se/vitamin E deficiency in ruminants), retained placenta and metritis (affecting about 9% of USA dairy industry births), and mastitis (estimated annual cost is \$17,500 for a 100-cow herd) (Surai, 2006).

The amount of Se available for the grazing beef cattle is dependent on Se content of the forages (McDowell, 1996). The concentration and availability of Se in

the soil determines the Se content of forages and the Se requirement for beef cattle among all ages is 0.10 mg Se/kg of diet (NRC, 2000). The Se content in forages (and grains) of the southeast USA (including Kentucky) is low (< 0.05 mg Se/kg) to variable (< 0.1 mg/kg) (Ammermann et al., 1975). Consistently, a survey of whole blood Se concentrations of beef cows and heifers from 253 cow-calf operations in 18 states (Dargatz and Ross, 1996) found that 42% of southeastern USA (including Kentucky) cattle were Se deficient ($\leq 0.080 \mu\text{g/mL}$), as opposed to 18% nationally. Therefore, dietary supplementation of Se is necessary in Kentucky.

Se supplementation is regulated by the Food and Drug Administration and cannot exceed 120 mg Se/kg or an intake of 3 mg per day when delivered in free-choice mineral supplements to cattle (FDA, 1987). Se can be added to diets in either inorganic or organic forms. Historically, the supplemental inorganic forms of Se typically used are sodium selenite or sodium selenate (Brennan et al., 2011), despite the knowledge that Se primarily is present in plants as organic forms. Organic forms of Se include selenomethionine (SeMet) and selenocysteine (SeCys), which are typically added to ruminant diets as constituents of Se-enrich yeast extracts (e.g., SeMet is the predominant form of Se in Sel-Plex[®] (Alltech Biotechnologies, Inc., Nicholasville, KY) (Korhola et al., 1986).

Even though they have different delivery speeds to liver (Kazuo and Suzuki, 2005), few differences in glutathione peroxidase (GSH-Px) activities and Se concentrations in blood and milk result from feeding selenite versus selenate to heifers (Ortman et al., 1999). However, it may be reasonable to expect few differences as selenate is probably converted to selenite in the rumen (Weiss, 2003).

The effect of supplementing diets with inorganic (ISe) versus organic (OSe) forms of Se on Se bioavailability and bioactivity also has been compared.

Assimilation of Se after 105-106 days by whole blood, red blood cells, and biopsied liver tissue was greater for heifers fed 3 mg/day of Se as Sel-Plex[®] versus sodium selenite (Liao et al., 2011). Similarly, Se concentrations (219 to 257 µg/kg wet weight) in the liver of cows supplemented with 3 mg/day as Se-yeast (Sel-Plex 50[®]) was 1.2-1.5 fold higher than with sodium selenite (Ortman and Pehrson, 1997). Thus, the bioavailability (defined as blood and tissue Se concentrations) of Se from OSe sources is higher than the ISe sources.

Regarding production responses to feeding OSe versus ISe, few studies have found major differences between average daily gain, average daily feed intake, or gain: feed ratios, or any other production performance parameter (Davis et al., 2008; Liao 2011; Nicholson, et al., 1991; Gunter et al., 2003). However, feeding organic forms of Se to dams may have positive effects on their offspring. For example, feedlot steers (Clyburn et al., 2007) and calves born from OSe-supplemented cows (Guyot et al., 2006) tend to have better average daily gain compared to calves from ISe supplemented cows. As for the Se biopotency (GSH-Px activity), different experiments on beef cattle have demonstrated that supplementation of OSe (as Se yeast) will increase blood Se GSH-Px versus ISe (sodium selenite) for weaned beef steers (Fry et al., 2005; Gunter et al., 2003; Guyot, et al., 2007; Nicholson, et al., 1991). This finding indicates that the Se biopotency is higher for beef cattle if dietary OSe is supplemented versus ISe. Similarly, with regard to the animal responses under stress condition including animal immunocompetence, OSe supplementation is reported to induce higher states of immunocompetence than ISe, including increased macrophage phagocytosis (Beck et al., 2005), higher production of antibodies in response to antigen after infection (Nicholson et al., 1993), and decreased mean milk somatic cell counts (Harrison et al., 2005). Such positive results in biopotency and

immunocompetency with OSe versus ISe fed cattle, have led some to recommend that OSe replace ISe forms of Se in cattle diets (Surai, 2006).

Recently a formulation of mixed (1:1) inorganic-organic Se supplements has been used in beef cattle mineral mixes as the source of Se (University of Kentucky Beef Cattle Mineral Mix). However, how this “mix” affects tissue Se assimilation and metabolism relative to OSe or ISe Se sources is not known. To initiate such comparisons, the diet of beef heifers was supplemented with none (Control) or 3.0 mg Se/day as ISe, OSe (Sel-Plex[®]), or Mix (1.5 mg Se/day as ISe:1.5 mg Se/day as OSe) for 224 days (Brennan et al., 2011). More Se was found in whole blood, red blood cells, serum, and liver of Mix and OSe heifers than ISe heifers, and all Se supplementation treatments resulted in greater Se assimilation than for non-supplemented Control heifers. As stated by the authors, from a biochemical perspective, the observed differences in steady-state Se assimilation by tissues resulting from consumption of OSe versus ISe likely reflects the known common and different metabolic fates of organic and inorganic Se forms and, by extension, may provide insight into why consumption of a mix of 1.5 mg OSe and 1.5 mg of ISe resulted in liver assimilation of Se that was equal to consumption of 3 mg/day of OSe and greater than ISe (Brennan et al., 2011).

To better understand effects of different forms of Se supplementation of beef cattle diets on whole-animal parameters and Se-specific metabolism, knowledge about how the forms of supplemental Se may affect global gene expression profiles is critical. However, with the exception of a pilot study by this research group (Liao et al., 2010), no research has been conducted to determine the effect of dietary Se supplementation on gene expression by beef cattle. The Liao et al. (2010) experiment revealed that expression of about 80 genes by the liver of slow maturing beef heifers

was altered by daily supplementation (105-106 days) of diets with 3 mg Se/day of ISe or OSe versus non-supplemented, but Se adequate, cohorts. Because, however, only a single time point was used in the experiment, it was not known if these differences reflected stable or transient responses to forms of Se supplement.

To address this issue, and to determine the effect of a “mixed” Se supplement consisting of 1:1 sodium selenite: Sel-Plex[®] (Mix), an expanded experiment (Brennan et al., 2011) using the same animal model (maturing beef heifers and cottonseed hull-based diet) was conducted over a 224-day period using 3 mg Se/day dietary supplement treatments (n=10) of ISe, Sel-Plex[®], or Mix. Importantly, this study found that liver Se concentrations were plateaued and stable after 112 days of supplementation.

Thesis objective

Therefore, using day 168 liver tissue samples from this experiment (Brennan et al., 2011) and microarray methodologies, the first objective was to test the hypothesis that the hepatic transcriptome profiles identified in biopsied liver samples from maturing beef heifers would be affected by the form of supplemental Se consumed. The secondary objective was to conduct bioinformatic analyses of these profiles to predict ostensible changes in physiological capacities induced by feeding different forms of supplemental Se.

Chapter 2: Literature Review

Se specification

Selenium exists as inorganic and organic forms (Foster and Sumar, 1997). The Se ion is conserved in minerals and soils (Underwood and Suttle, 2000). Plants assimilate inorganic Se from soils and convert Se it into inorganic and organic forms (Finley, 2005). Thus, the amount of Se contained in forages dictates the Se status of grazing animals.

As mentioned in the Introduction, the Se requirement for beef cattle is 0.10 mg Se/kg of diet (NRC, 2000). In Kentucky with its Se-deficient soils, the predominant forage is tall fescue and the average Se content of tall fescue is 0.06 mg/kg (John et al., 2003). Thus, Kentucky cattle typically consume Se-inadequate forages.

Plants can be divided into two groups with regard to Se. One is called “non-Se-accumulating”, and the major Se species in this group are selenate and SeMet, plus smaller amounts of SeCys (Jacobs, 1989). The other group is known as “Se-accumulating”, and the predominant form of Se is γ -glutamyl methylSeCys (Shrift and Virupaksha, 1965; Terry et al., 2000). From recent findings, the major forms of Se available in feedstuffs for animals are SeMet and SeCys (Huerta et al., 2004). SeMet is predominant in cereals (Fairweather-Tait et al., 2010). Many plants contain plentiful SeMet because they have proteins that are high in sulfur-containing amino acids, and SeMet can nonspecifically replace Met in binding to tRNA_{met} (Fairweather-Tait et al., 2010). SeMet also is the main Se compound in yeast and has been identified after enzymatic digestion or acid hydrolysis (Gammelgaard et al., 2008).

Plants take up huge amounts of Se from the soil and transform it through several biochemical steps into volatile species, a process called phytovolatilization (Dumont et al., 2006). DMSe (Dimethyl Selenide) is the main phytovolatilization

product (Dumont et al., 2006). SeMet is favored for volatilization process, however, SeCys is incorporated into proteins, hence, is no longer available for volatilization. Inorganic Se from soils can be incorporated into the SeCys by plants (Ellis and Salt, 2003). After incorporation, the SeCys can be incorporated into Se-proteins. The SeCys can also be metabolized into various non-proteinogenic Se amino acids (Neuhierl et al., 1999). Their synthesis occurs along the S-pathway (Dumont et al., 2006). Three metabolites: Se-MeSeCys, Se-Cystathionine and γ -glu-SeMeSeCys are converted from the SeCys. The formation of Se-Cystathionine results in its accumulation of it because the enzyme cystathionine β -lyase is unable to cleave this Se analogue (Terry et al., 2000).

Se deficiency

It has been reported that Se deficiency is associated with physiological discomfort and diseases in ruminant animals, including nutritional muscular dystrophy and white muscle disease (the most well documented disease related to Se/vitamin E deficiency in ruminants), retained placenta (retained fetal membranes) and metritis (affecting about 9% of all calvings in US dairy industry births), mastitis (costs \$100-200/cow/year or about \$17,500 annually for an average 100-cow herd), unthriftiness and reduced growth rate, reduced reproduction rates, immunosuppression and increased susceptibility to various disease, and sub-optimal productivity and early mortality (Surai, 2006; Pehrson, 1993; Kolb and Seehawer, 2001). Thus, the maintenance of Se in an adequate level for the beef cattle is critical.

Se requirements for cattle

It is recommended that diets consumed by beef cattle at all stages of life (calves, heifers and lactating and dry cattle) should be 0.10 mg Se/kg of diet, and that

the maximal amount of supplemental Se not exceed 3.0 mg Se per day (NRC, 2000). The Se level in cattle has been classified as adequate, marginal and deficient when Se level in plasma/serum were >75, 50-75 and <50 ng/ml; in whole blood >200; 140-200 and <140 ng/ml, in liver >1.25, 0.6-1.25 and < 0.5 ng/mg dry matter, respectively (Smith et al., 1998; Zarski et al., 1998; Kincaid, 1999). During some specific periods such as reproductive period, the requirement of Se is increased, thus the original Se sources cannot meet the Se requirement of cattle and additional Se supplement is necessary (Maus et al., 1980).

Se availability for cattle

The amount of Se available for grazing beef cattle is dependent on the forages (FDA, 2007). As the plant Se is originally from inorganic Se in the soil, the concentration and availability of Se in the soil determine the Se content of forages (Hintze et al., 2001). For the areas where soil Se is deficient, beef cattle are more easily subjected to Se deficiency. The geographic distribution of Se in forages and grains, the southeast USA (including Kentucky) have low (80% of all forage and grain contain < 0.05 mg Se/kg) to variable (50% of all forage and grain contain < 0.1 mg/kg) Se (Juniper et al., 2008). Forty-two percent of cattle in the southeastern United States (including Kentucky) were Se deficient ($\leq 0.080 \mu\text{g/mL}$) compared to 18% of deficient animals nationally after testing whole blood Se concentrations of cattle and heifers from 253 cattle-calf operations in 18 states (Dargatz and Ross, 1996). Thus, the supplementation of Se for beef cattle is important in geographical areas where Se is not adequately available for cattle, including Kentucky.

Se supplementation

Mineral supplements for feedlot cattle are incorporated into their concentrate diets. However, cattle grazing Se-deficient feedstuffs can receive mineral supplements through a variety of delivery systems (Wichtel, 1998; Hemingway, 2003). These include licks, drenching with Se compounds, intraruminal boluses, selenite or selenate injections, depot injections, adding sodium selenite to the drinking water, various methods of pasture and soil application, and by adding Se into feedstuffs (McDowell, 1996; Pavlata et al., 2001). Dietary Se supplements added in feedstuffs can be further divided into organic and inorganic according to their chemical forms. Inorganic Se supplements are frequently used and treated as an ordinary Se supplements for ruminant (Azzi et al., 2005). However, irrespective of methods of Se supplementation used, Se inadequacy in beef cattle is still a global problem. This is partly due to usage of inorganic forms of Se with low Se availability. Therefore, how to supply Se in more available is an ongoing area of research. Organic Se can substantially improve Se status of beef cattle and research is still ongoing to define out how to optimally relieve the Se deficiency by organic Se. For this reason, and to understand whether inorganic vs organic forms of dietary Se differentially affect cell function, it's crucial to understand Se metabolism.

Se Metabolism

Selenium metabolism consists of absorption, transfer, incorporation into body proteins, and excretion of Se. In mammals, organic and inorganic Se forms follow different metabolic pathways, although there are overlapping components. This metabolism is affected by such factors as the chemical form of Se, level of Se in

blood and tissues before supplementation, presence of various minerals and amino acids in the diet, and concentration of Se in the diet (Thomson, 1998).

Absorption

The absorptive mechanisms for Se have not been fully characterized. Several factors can affect efficiency of Se absorption, including the form of the element, the amount that was ingested, and other dietary factors such as calcium, arsenic, cobalt and sulfur can affect Se absorption by 50% or more (Surai, 2006). In general however, most Se is efficiently absorbed in the small intestine (Schrauzer, 2000), just over half is retained, (ie, not excreted in the urine) (Bugel et al., 2003), and subsequent metabolism depends on the form in which Se is present in plasma (Fairweather-Tait et al., 2010).

Balance trials with grower-finisher pigs evaluated sodium selenite vs Se-enriched yeast at various dietary Se levels (0.1, 0.3, or .5 ppm Se). As dietary Se levels increased, urinary Se increased more when pigs were fed sodium selenite (Mahan and Parrett, 1996). Selenium retention (a percentage of Se intake) was greater ($P = 0.01$) when organic Se (0.3 ppm of Se from Sel-Plex) was supplemented compared with inorganic Se (sodium selenite) to broilers for 6-wk (Yoon et al., 2007). Selenium retention in the liver, kidney, pancreas and loin of growing-finishing pigs was higher when pigs were fed organic Se (Se-enriched yeast) than the same amount of inorganic Se (selenite Se). (Tian et al., 2006)

Different forms of dietary Se also are absorbed at different rates. It was reported that selenate has the most efficient absorption, nearly 100%, but a significant fraction is lost in the urine; 90% of SeMet is absorbed; SeCys is absorbed very well; >50% of selenite is absorbed and is better retained than selenate (US Food and Nutrition Board, 2000). Even though selenate and selenite are more efficiently

absorbed, they are less retained vs the organic forms of Se, SeMet and SeCys (Ammerman and Miller, 1975; Thomson et al., 1978; Schrauzer, 2000; Burk et al., 2006). Thus, considering only tissue retention as a criterion, feeding organic forms of Se may be the more efficient method of supplementing Se to animal diets.

Blood Transfer

After absorption, Se is transferred in blood bound to alpha- and gamma-globulins and delivered to the liver to synthesize selenoproteins (Ceballos and Wittwer, 1996). It was reported that SeMet-Se is incorporated into a longer term body pool than the selenite-Se: It is steadily incorporated into erythrocytes during a period of 8-12 weeks and plasma Se reached a maximum 3-4 h after administration and about 4-8 h sooner than after the administration of an equivalent dose of selenite; selenite increased the blood until a plateau was reached after 7-8 weeks (Schrauzer, 2003).

Incorporation of Se into proteins

Selenium can be incorporated into proteins to form Se-containing proteins and selenoproteins. The Se-containing proteins are proteins either with non-specific incorporation of Se (usually SeMet) or specific Se-binding proteins. In contrast, selenoproteins are specific proteins containing Se in the form of genetically encoded SeCys (Behne and Kyriakopoulos, 2001; Almondes et al., 2010). Different from proteins that bind zinc and copper to form coordinated to functional groups, SeCys and SeMet residues are constituent residues in polypeptides (Suzuki, 2005).

Selenium-specific Metabolic Pathways

General metabolism

The metabolism of organic and inorganic forms of Se have both common and different metabolic fates. Selenate, selenite, SeMet, and SeCys all enter the selenide pool and from here the Se is either converted to selenophosphate and used for synthesis by specific tRNAs or excreted in the urine as a selenosugar (Schrauzer, 2003; Fairweather-Tait et al., 2010). Specifically, SeCys and SeMet are thought to be the most common forms of dietary OSe. In the ruminant, absorbed SeCys (originating from dietary or microbial protein), will be transformed to selenide by cleavage of the C-Se bond at the beta position by beta-lyase, this step is involved the production of alanine (Ohta and Suzuki, 2008). Besides, the methyltransferases can transfer one-carbon groups to SeCys to become methylSeCys, the later will be converted to gamma-glutamyl-Se-methyl-SeCys, methyl-seleno-pyruvate, or methylselenol that functions as an intermediate of Se excretion and selenoprotein synthesis. The selenide undergoes an ATP required reaction to synthesize the selenophosphate by selenophosphate synthetase. The transfer RNA for selenocysteine will be aminoacylated with serine to become $\text{tRNA}^{[\text{Ser}]}\text{Sec}$, then binds with the selenophosphate to form phosphoseryl-tRNA^[Ser]Sec by O-phosphoryl tRNA Sec kinase, and further converts to selenocysteyl-tRNA^[Ser]Sec by the Selenocysteine synthase. Along with some transcription factors, the selenocysteyl-tRNA^[Ser]Sec will bind to the UGA codon to synthesize the selenoproteins, however, SeMet can incorporate into polypeptide chains using the same mechanisms of Met to synthesize Se-containing proteins in whole body by the transfer RNA for Met, but it can also transfer to SeCys similar to how Met transfers to Cys to synthesize selenoproteins when the body is under a relative Se-deficiency environment. Also, SeMet can be

transformed to methylselenol through cleavage of the C-Se bond at the γ -position of SeMet and then the methylselenol is transformed to selenide.

Inorganic Se (selenite and selenate) are reduced to selenide by thioredoxin reductases, and the resulting selenide used to synthesize selenoproteins. Selenate can convert to selenite but this step differs from mammal compared to bacteria. In mammals, the bifunctional enzyme phosphoadenosine-phosphosulfate synthase will catalyze two steps. Sulfate adenylyltransferase catalyzes the formation of adenosine 5'-phosphosulfate (APS) from ATP and selenate, and APS is bound by adenylylsulfate kinase portion of 3'-phosphoadenosine 5'-phosphosulfate (PAPS) synthase to selenite (Bandurski et al., 1956; Hilz and Lipmann, 1955; Venkatachalam et al., 1998). In contrast, in bacteria, this reaction is carried out by selenate reductase (Schroder et al., 1997; Macy et al., 1993; Krafft et al., 2000; Stolz and Oremland, 1999). At the same time, selenite can also be converted back to selenic acid, and the latter adenylylated to adenylylselenate and then phosphorylated to phosphoadenylylselenate. Both of these steps are catalyzed by bifunctional phosphoadenosine-phosphosulfate synthase (Lehninger, 2005; Salway, 2004).

Oxidized glutathione (GSSG) can incorporate selenite to become GS-Se-SG, and the latter then reduced to GS-SeH by glutathione reductase. GS-SeH can reversibly convert to selenide upon GSH conversion to GSSG. GS-SeH, also will bind glycogen or carbohydrate group to produce the selenosugar A. Methylation of Selenosugar A (with SAM acting as the methyl group donor) produces selenosugar B. Selenosugar B is the principle Se metabolite when the body is in a low-toxic range of Se (Kobayashi et al., 2002). Selenide can be converted back to methylselenol by methylation, and the methylselenol will also be methylated into dimethylselenide, which will be further methylated into trimethylselenide. These three reactions

catalyzed by methyl transferase use S-adenosylmethionine (SAM) to donate the methyl group (Ganther et al., 1966; Hsieh and Ganther, 1977; Suzuki et al., 2006b). Methylselenol can be reduced to methyl-selenic acid through a reversible reaction with thioredoxin reductase (MOORE et al., 1964; Speranza et al., 1973; Arner and Holmgren, 2000). Toxic doses of Se is known to be excreted into urine in the form of trimethylselenide and exhaled in the form of dimethylselenide (Kobayashi et al., 2002).

The Se in inorganic selenite or selenate in the diet will either go into selenoprotein or be excreted in the form of methylated Se or selenosugar. However, Se in the organic form SeMet can be preserved in Se-containing proteins except for synthesis of selenoprotein or excretion. The preserved Se from organic Se will be used once the body is Se deficient or the requirement of Se is increased under stress conditions (Surai, 2002).

Selenocysteine (SeCys)

SeCys is directly incorporated into the polypeptide chains of selenoproteins (B öck et al., 1991). The existence of SeCys rather than cysteine increases the enzymatic activity of selenoproteins as much as 100- to 1,000-fold (Burk, 2002). Organic Se compounds are transformed to selenide through reductive cleavage of the C-Se bond by lyase reactions (Suzuki, 2005). In contrast to Cys, SeCys residues from selenoprotein degradation are not appreciably re-incorporated into proteins. Instead, they are degraded in the liver to selenide by β -lyase, with the Cys residue re-utilized for protein synthesis. Selenide is phosphorylated to selenophosphate, which will be transformed to SeCys on SeCys-tRNA (21st aminoacyl-tRNA), and the selenocysteinyl residue is incorporated into selenoprotein sequences.

Incorporation of SeCys into selenoproteins requires five specific components. They are: a SeCys-insertion sequence element (SECIS) in the 3'-untranslated region, a SeCys codon (UGA) in the coding region, a SeCys-specific translation elongation factor, the SeCysSecCystRNA, and a SECIS-binding protein (SBP2) (Suzuki, 2005; Daniel and William, 2008).). The initial step in biosynthesis is the charging of serine on the specific tRNA^{Sec} by seryl-tRNA synthetase, followed by Ser-tRNA^{Sec} to Sec-tRNA^{Sec} conversion, which is catalyzed by SeCys synthase, an enzyme that utilizes phosphoserine as an intermediate to generate SeCys (Sturchler-Pierrat, et al., 1995). The whole process included three different steps: aminoacylation of tRNA^{Sec} with serine by seryl-tRNA synthetase; transformation of serine to phosphoserine; and conversion of phosphoserine to SeCys. The SECIS (SeCys insertion sequence) element is an RNA stem-loop structure that resides in the 3'UTR of selenoprotein mRNA. This SECIS element is mandatory for UGA recognition as Sec and not a stop codon (Aeby et al., 2009). A stem loop structure that resides 7 nucleotides distant 3' to the UGA Sec codon is the Se response element (SRE). Presumably, the SRE can stimulate decoding of UGA (Nasim et al., 2000).

A number of protein factors also are essential for selenoprotein synthesis. For example, EFSec (which binds only to the Sec-tRNA^{Sec}) acts as the elongation factor for selenoprotein B (SelB) and nucleolin and NSEP1 are cofactors that bind SECIS (Lescure et al., 2002).

Selenomethione (SeMet)

The incorporation of SeCys into selenoproteins is known as the “regulated pathway” of Se incorporation into proteins because of the obligate SECIS genetic element (Suzuki, 2005). In contrast, the incorporation of Se into polypeptide chains through SeMet is known as the “unregulated” pathway (Papp et al., 2007) because

SeMet is directly incorporated into polypeptide chains. Furthermore, tRNA_{Met} readily recognizes SeMet and does not distinguish between SeMet and Met (Cowie et al., 1959; Burk et al., 2001; Suzuki, 2005). Because SeMet and Met share the same codon, the ratio of incorporation into proteins for SeMet is dependent on the SeMet:Met ratio in feedstuffs, and excessive SeMet supplementation increases this ratio and the incorporation content. Once proteins are synthesized, SeMet is retained at the pre-synthesis ratio of SeMet:Met (Suzuki, 2005). Once SeMet is metabolized as a constituent of the Met pool, it is available to be incorporated randomly into cellular proteins and is unaffected by specific Se-metabolism processes (Burk et al. 2001). SeMet, however, can be metabolized to selenide through the trans-selenation pathway or through the direct lyase reaction. Thus, the non-specific incorporation of SeMet in place of Met in various proteins is a way of preserving Se for future use in the body (Zeng, 2009; Hall et al., 2012).

The Se in SeMet can become available for regulated pathway-mediated selenoprotein synthesis when catabolized to selenide by γ -lyase, especially during periods of excessive SeMet intake (Behne, et al., 1992; Schrauzer, 2003). Then it shares the exact same pathway to synthesize selenoproteins as SeCys. Gamma-lyase activity also can yield methylselenol (CH₃SeH), which has been identified as an anti-carcinogenic agent from SeMet produced selenide, whereas β -lyase produces Se from MeSeCys during SeCys metabolism (Suzuki, 2005). Methylselenol is primarily excreted in breath and urine after conversion to TMSe (Gabel-Jensen et al., 2010) but may also enter the selenide pool via the reverse reaction by demethylase (Fairweather-Tait et al., 2010). The presence of γ -lyases in the liver suggests that the liver is the primary site of SeMet degradation.

Inorganic Se

The inorganic Se compounds selenite and selenate are commonly reduced to selenide for further utilization or excretion, but their primary routes of metabolism differ (Suzuki, 2005). For example, blood selenite is readily absorbed by red blood cells (RBCs) whereas selenate ions are absorbed by hepatocytes through a transport system for phosphate or excreted directly into urine (Suzuki, 2005). After absorption by RBCs, selenite is readily reduced to selenide, effluxed into blood, and bound by albumin for transfer to the liver. Subsequently, selenide and selenate are used by the liver for synthesis of selenoproteins through incorporation into SeCys (Suzuki, 2005), as described above.

Selenoproteins

Selenoproteins are defined as proteins which require SeCys incorporation into their polypeptide chains. There are 25 selenoprotein genes in humans, and SeCys residues were found in the active site of those that have been attributed a function (Allmang et al., 2009). Most selenoproteins function as peroxidases. The first mammalian protein identified as a selenoprotein was cytosolic glutathione peroxidase (GPX-1) (Allan et al., 1999). Additional glutathione peroxidase enzymes have been identified, including a glycosylated plasma glutathione peroxidase (GPX-2), a gastrointestinal glutathione peroxidase (GPX-3), and a phospholipid-hydroperoxide glutathione peroxidase (GPX-4) (Tham et al., 1998). Except for GPX-4, these peroxidases exist as tetramers, with each subunit containing one SeCys residue (Allan et al., 1999). The tetrameric forms catalyze the reduction of a variety of hydroperoxides, including hydrogen peroxide, cumene hydroperoxide, t-butyl-hydroperoxide, and fatty acid hydroperoxides (Flohe 1989; Wiley et al., 1995). In contrast, GPX-4, a monomer that contains one SeCys residue, catalyzes the reduction of both fatty acid hydroperoxides and cholesterol hydroperoxides (Ursini et al., 1985).

After reaction with a peroxide substrate, regeneration of the reduced active form of the enzyme requires GSH (reduced glutathione). These selenoproteins have anti-oxidative activities in the body, and glutathione peroxidase and thioredoxin reductase are thought to be the most abundant antioxidant enzymes in mammals (Gladyshev et al., 1998).

Several selenoproteins, including selenoprotein P and W, and GPx 1, 3, and 4, have been used widely as biomarkers of Se status (Brown and Arthur, 2001). However, because selenoprotein P typically accounts for approximately half of the Se in plasma, reaches a plateau after 2-4 weeks of supplementation, and is well correlated with plasma Se across a wide range of Se status, it is often considered to be the best biomarker of Se status (Fairweather-Tait, et al., 2010).

Excretion of Se

Selenium is excreted in urine (50 – 60%), in feces, and expired. However, Se expiration only occurs when Se intake is extremely high (Suzuki, 2005). If toxic levels of Se are not absorbed, the major urinary metabolite of Se is selenosugar. Two different Se sugars have been identified: Se-glutathionyl-N-acetylselenohexosamine (selenosugar-A) and Se-methyl-N-acetylselenohexosamine (selenosugar-B) (Vadhanavikit et al., 1993; Kobayashi et al., 2002; Gammelgaard et al., 2008). Selenosugar-A is thought to be the precursor for selenosugar B. Selenosugars are thought to be produced principally by the liver, absorbed by kidney, then excreted into urine (Suzuki, 2005).

However, if excessive Se is absorbed, it is excreted as methylated selenide in the urine, mon-, di- (DMSe) and trimethylselenonium (TMSe) (Ali and Aboul-Enein, 2006). In addition, DMSe is known to be exhaled (Vadhanavikit et al., 1993). The ratio of the two major Se metabolites in urine changes depending on the Se dose, i.e.,

at a lower dose, Se is excreted mostly as monomethylated Se, whereas TMSe is the primary form of excretion when high levels of Se are consumed (Suzuki, 2005).

Toxicological and Nutritional Markers

Consumption of Se at or above a concentration of 2.0 µg/g diet or mL of drinking water are toxic (Suzuki, 2005). As discussed above, TMSe becomes the predominant form of Se excretion at these levels, at least in rats (Kobayashi et al., 2002). Thus, selenosugars are assumed to be a nutritional marker for non-toxic Se consumption whereas TMSe may be a toxicological marker (Kobayashi et al., 2002).

Ruminant Se Metabolism

Metabolism of Se by ruminants has common and uncommon components with nonruminants.

Absorption and transfer

It has been reported that ⁷⁵Se, as a selenic salt, was unabsorbed from the rumen, slightly absorbed from the abomasum, secreted into duodenum and jejunum with net absorption by the ileum (Wright and Bell, 1996). The absorbed Se was either bound to carrier proteins, or existed as a free form, for transport to the liver and other tissues. After absorption, Se metabolism in ruminants is similar to non-ruminants (Ceballos and Wittwer, 1996).

Ruminant vs Nonruminant Se metabolism

The absorption and availability of inorganic Se is very low for cattle, with only about 11 – 16% consumed being absorbed (Koenig et al., 1991; Koenig et al., 1997) and about only 14% of that fed (136.9 of 973.3 µg/daily Se) was accumulated in the cattle body (Kamada et al., 1998). Accordingly, absorption of inorganic Se in ruminants is much lower than in monogastric animals. That is, absorption of orally

administered ^{75}Se for sheep was less than half that by swine and rats (34% vs 85%) (Wright and Bell, 1966; Mason and Weaver, 1986), and the low net absorption and bioavailability of Se for ruminants has been attributed to Se metabolism by rumen bacteria (Kamada et al., 1998).

One reason for this low absorption of inorganic Se for ruminants may be the Se-reducing conditions of the rumen and microorganisms influence. That is, rumen bacterial convert inorganic Se into insoluble forms such as metallic Se or selenides (Peterson and Spedding, 1963; Cousins and Cairney, 1961; Spears, 2003), which results in decreased rates of absorption. The fate of inorganic Se that is absorbed by microorganisms, is incorporation into microbial proteins (presumably as SeCys), thus providing a source of organic Se for digestion and absorption by the ruminant digestive tract. It has been shown that Se concentrations in bacteria isolated from sheep rumen is 2.3 and 3.9 times the original dietary Se concentration of the forage and concentrate diets, respectively (Koenig et al., 1997). Likewise, Se in the liquid phase of digesta was largely protein-bound and, following hydrolysis of the microbial cell protein, the element was absorbed as Se-containing free amino acids (Hidioglou and Jenkins, 1974). It is known that 6 h post dosing, 50% of Se was present in rumen bacterial protein (Hidioglou and Jenkins, 1974).

Different species of bacteria in the rumen metabolize inorganic Se into different forms. For example, *Selenomonas ruminantium* can accumulate selenite into SeCys, selenoethionine, SeMet and red elemental Se. However, some bacteria such as *Bacteroides ruminicola* cannot incorporate selenite into organic compounds (Hudmann and Glenn, 1984; 1985). In addition, the overall availability of inorganic Se incorporated into bacterial proteins is very low, which means that a lot of inorganic Se is unavailable for ruminant metabolisms. In contrast, the organic Se containing

amino acids can be used for available Se directly. From this perspective, organic Se is more bioavailable for for ruminants than inorganic forms.

The effect of Se supplementation on rumen bacterial fermentation

Dietary Se supplementation can affect rumen conditions and metabolism, especially rumen microbial fermentation (Kim et al., 1997). It was reported that rumen gas production was positively correlated with supplemented Se between 0.2 and 0.4 mg Se/kg dietary dry matter, and short chain fatty acid production was optimal at 0.2 and 0.4 mg Se/kg dry matter but decreased significantly for concentrations greater than 12.8 mg Se/kg dry matter (Von Brehm, 2001).

Excretion

Excretion of Se by cattle is through urine, feces, and exhalation. When Se is administered via intravenous or subcutaneous injection of ruminants, urine is the major route of excretion (Wright and Bell, 1966). The other principal route of Se excretion in ruminants is the feces (Wichetel, 1998; Neathery et al., 1990; Podoll et al., 1992).

Tissue specific Assimilation of Se

In mammals, Se is incorporated into red blood cells at time of their formation; therefore, taking into account their life span (90-120 days) Se content of erythrocytes reflects Se intake 1-3 months previous (Smith et al., 1997; Smith et al., 1998). It is generally accepted that Se concentration in serum or plasma reflects short-term Se nutritional status, whereas whole blood (erythrocyte) Se and GSH-Px activity are more indicative of long-term Se status (Thompson et al., 1998).

Se preferentially accumulates in tissues such as endocrine glands, brain, and reproductive organs (Allan et al., 1999). In the liver, dietary Se is metabolized to selenide and then incorporated as SeCys into selenoprotein P, the physiological form

that distributes Se from the liver to peripheral tissues and other organs (Dumont et al., 2006). The kidney can synthesize the other major plasma selenoprotein, GPX3. In the liver and kidney, cellular GPx was detected as the carrier of endogenous Se (Suzuki and Ogra, 2002). Many tissues can indiscriminately incorporate SeMet into proteins in competition with Met, and both the liver and extra-hepatic tissues have been predicted to metabolize SeMet to multiple end products with the potential for biological activity. The inter-tissue (liver, kidney, serum, and urine) distribution of selenite and selenate Se was determined after intravenous injection (Suzuki and Ogra, 2002). Red blood cells take up selenite and selenite is reduced by glutathione to selenide, which is transported to plasma, selectively bound to albumin, and transferred to the liver (Shiobara and Suzuki, 1998).

Manipulation of Inorganic Versus Organic Se Metabolism

Organic versus inorganic Se tissue assimilation

It is generally accepted that tissue assimilation of organic Se is greater than assimilation of inorganic Se in cattle. Serum Se concentrations tended to be uniformly higher during and at the end of the trial in beef cattle given Se yeast compared to selenite (Fisher, 1995; Fisher et al., 1995). The supplementation of organic versus inorganic Se (0.2 mg/day) for 8 weeks to Se-deficient cattle resulted in increased blood Se of 5.6 to 167 (Se-yeast) per $\mu\text{g/L}$ versus 91 $\mu\text{g/L}$ (selenite) (Malbe et al., 1995). Selenium (0.75 mg daily) from the Se-yeast maintained Se concentrations in whole blood and milk at the same levels as Se 3.0 mg in the form of sodium selenite and Se (3.0 mg) from the yeast product increased blood Se by 40% and that in milk by 100%, compared to the equivalent amount of inorganic selenite (Ortman and Pehrson, 1997). The Se concentration in plasma was 1.26 fold higher for heifers supplemented with Se-yeast compared to sodium selenite (Weiss, 2003). Furthermore, a 20 to 30%

increase in whole blood Se levels was associated with organic Se supplementation (Sel-Plex, Alltech, Inc.) compared to inorganic Se (Elliott et al., 2005).

Organic Se versus inorganic Se on Se bioavailability

Because serum Se concentration can reflect the GSH-Px activity of beef cattle, the serum Se concentration was used as the value to measure the bioavailability of Se in beef cattle. It should be mentioned that dietary Se is highly correlated to GSH-Px when animals are deficient in Se, but the relationship weakens as animals become adequate in Se (Stadmore et al., 1982; Combs and Combs, 1984; Kincaid, 1999; Rock et al., 2001). Thus, after reaching a certain concentration of Se in the blood, which is necessary for maximum expression of GSH-Px, there is no further increase in the enzymatic activity of GSH-Px.

In general, the bioavailability of organic Se was superior to inorganic Se. The relative bioavailability (selenite=1) of yeast Se was 1.4 if blood GSH-Px, 1.9 if blood Se, and 2.7 if milk Se was used as the response criterion (Malbe et al., 1995). The bioavailability of Se (GPx activity in the erythrocytes) for Se-deficient heifers supplemented with Se-containing yeast for 12 weeks was about twice that than for heifers supplemented with equal amounts of inorganic sources (Na or Co selenite) (Pehrson et al., 1989).

Genotype can also affect the Se bioavailability. The Se bioavailability response by humans after 6 wk of Se supplementation with 100 µg sodium selenite/d was influenced by genetic polymorphisms in the selenoprotein P (SEPP) and GPX4 genes. These biomarkers (plasma Se, selenoprotein P, and GPx3) that used to assess Se bioavailability were associated with 2 common single nucleotide polymorphisms in SEPP in both baseline and post-supplementation samples, and the GPX4 polymorphism was shown to influence lymphocyte GPx4 concentration and other

selenoproteins in vivo (Meplan et al., 2007). A single nucleotide polymorphism in GPx1 was associated with Se deficiency and impaired GPx1 activity (Lei et al., 2009) and also may be associated with a different response of GPx1 activity to Se (Jablonska et al., 2009). It is possible that common polymorphisms in selenoprotein genes, such as SEPP, GPX1, GPX4, and selenoprotein S (SELS) will have a significant effect on the metabolism of dietary Se and will generate differences in bioavailability. This possibility warrants further investigation.

Organic versus inorganic transfer of Se into colostrum and milk

It is well known that cattle have a limited ability to transfer Se into colostrum and milk when sodium selenite is used as a dietary supplement. It was found that organic Se in the ruminant diet contributes to higher Se concentration in colostrum and milk, which could help to build antioxidant system of newborn calves through the antioxidant activity of Se (Slavik et al., 2008).

It was reported that Sel-Plex supplemented diets had 67% higher milk Se content within 2 weeks of initiating supplementation than when sodium selenite was supplemented. Furthermore, somatic cell counts were significantly reduced 30% as early as 2 weeks after inclusion of Sel-Plex, and that this trend continued throughout the 6 week supplement period (Duarte et al., 2004). Similarly, inclusion of Se (0.2 ppm) in the form of sodium selenite (0.2 ppm) into the cattle diets provided 2.2 mg Se/day for 8 weeks was associated with an increased Se level in milk (0.138 mg/L versus 0.048 mg/L) and decreased somatic cell counts (174,500 versus 229,300) cells/mL (Foltys et al., 2004). The inclusion of Sel-Plex Se in cattle diets at 2 or 6 mg Se/day was associated with increased Se concentration in the milk from 6.9 up to 15.0 and 25.2 ng/ml, respectively, compared to inorganic Se (McIntosh and Royle, 2002).

The replacement of sodium selenite with Sel-Plex also has been associated with a significant increase in Se concentration in colostrum from 2.67 to 6 mg/day, and was associated with an increase in Se concentration in colostrum after the first two milkings by 42%, and after the first 8 milkings by 35% (Lewis, 2004). Others found that only 4.8% of Se was transferred to milk if sodium selenite was added, whereas 19% of the Se appeared in milk if organic Se was fed (Waite et al., 1975; Conrad and Moxon, 1979) and that the efficiency of Se transfer to milk of cattle fed Se-yeast ranged from 9.9 to 12.5%, compared with 2.4-4.1% for cattle fed sodium selenite (Givens et al., 2004).

Differential Organic Versus Inorganic Se Form Effects on Immune and Health Status

Many experiments have reported that organic Se supplement can contribute to higher Se concentrations in blood, liver, and milk, accompanied by improvement of animal quality, in particular with improved immunity and health. It was shown that organic Se supplementation of cattle diets could maintain high Se status during all periods of ontogenesis and to maintain high productive and reproductive performances in stress conditions of commercial meat and milk production (Lyons et al., 2007). Experimental data have included a number of observations such as: increased GSH-Px activity in erythrocytes, decreased somatic cell counts, improved health, and production and reproduction parameters in cattle supplemented with organic Se (Cortinhas et al., 2010).

During stress, the activity of proteasomes increase to provide amino acids needed for the formation of immune defense cells and enzymes, including Se-dependent GSH-Px and thioredoxin reductases (Surai, 2006). During such times, SeMet in proteins can be released by protein catabolism, thus providing a source of Se needed for synthesis of GSH-Px and other selenoenzymes. Because the SeMet can be

incorporated as “Met” through the non-regulated pathway, SeMet can be re-used (Suzuki, 2005).

The advantages of organic Se supplementation compared to inorganic Se include: 1. higher concentration of Se in the blood, milk, and tissues of cattle with Se-yeast compared to inorganic Se supplement; 2. long-term supplementation of cattle diets with Se-yeast does not result in toxic accumulations of Se, 3. beef calves whose dams were supplemented with Se-yeast had a higher Se status than calves whose dams were supplemented with selenite (Surai, 2006); 4. Se-yeast proved to be more effective in stimulating weight gains and liver Se concentrations than sodium selenite (Surai, 2006); 5. Se-yeast was more effective than sodium selenite in raising and maintaining adequate Se concentrations in tissues of beef cattle (Ortmana, 1999; Valle, 2001).

Overall, dietary Se supplementation in inorganic forms does not appear adequate to meet high Se demand of growing, reproducing, and lactating animals, and is less than adequate for further metabolism due to reduction to metallic Se or selenide by rumen bacteria and much lower retention level than organic Se. The feeding of organic Se, such as selenized yeast, results in increased Se concentrations in blood and GSH-Px activity. For example, Se concentration is doubled in colostrum and milk, and the Se transfer through placenta is greater when Se supplied as OSe than ISe (Surai, 2006). As a result, cattle health is improved with lower somatic cell counts, decreased mastitis and retained placenta and improved conception rates. The benefit to newborn calves is due to improved antioxidant defense and thermoregulation leading to better immunity, viability and lower mortality during first months of the postnatal development. It is also beneficial that organic Se SeMet can build Se reserves in their tissues, in particular in muscles and those reserves can be effectively

used by cattle in stress conditions when Se requirement is increasing while feed consumption is declining (Surai, 2006).

Effect of Se supplements on Aspects of Beef Cattle Physiology

The effect of supplementation of Se for beef on blood Se concentration and GSH-Px activity, selenium status in their calves, and immune status has been studied. Supplementation of Se has higher Se concentration and GSH-Px activity than non-supplemented beef. Cattle provided supplemental 2.5 mg/d Na selenite or Se-yeast (Sel-Plex; 2.5 mg/ d) had greater liver and plasma Se concentrations and greater plasma GSH-Px activity compared with control steers on day 60 and 90 (Arthington, 2008). Copper and Se supplementation to cattle maintained GSH-Px activity within normal range, compared to the non-supplemented animals that had GSH-Px activity was below the normal range (Minatel et al., 2002). Both 1.7 mg supplemental Se/d as sodium selenite and Se yeast increased blood Se concentrations of beef calves compared to the non-supplemented group (Fry et al., 2005).

Selenium content was positively correlated with the GSH-Px activity and antioxidative ability. The mean value of Se concentration in whole blood and GSH-Px activity of calves fed a basal diet and a ready-made fodder mix that contained 0.1 mg.kg⁽⁻¹⁾ selenium were higher than the non-supplemented group and was correlated with Se concentration in whole blood and GSH-Px activity (Harapin et al., 2000). Examination of blood serum and meat samples of clinically healthy Limousine and Angus calves grazed on grass pasture was negatively correlated with blood serum repetitive strain injuries and selenium content, which means a positive effect of Se content in beef on the protective antioxidative processes in blood serum (Matthes et al., 2002). However, even though supplementation of Se leads to increased serum and

tissue Se concentrations, these differences may not be concomitant with altered animal performance (Richards and Loveday, 2004).

Supplementation of beef cows with Se can maintain Se content and GPH-Px activity and immune response for their calves. At 180 d after birth, blood Se concentrations for calves of dams subject to no Se supplementation during day 115-130 of gestation were deficient ($<50 \mu\text{g Se/L}$), but Se yeast supplementation maintain an adequate Se level ($188 \mu\text{g of Se/L}$), and the liver Se content of calves was greater of supplemented group than control group (Davis et al., 2005). Supplementation with Se of pre-weaning calves increased whole blood Se concentration and GSH-Px and tended to increase in vivo cell-mediated immune response compared to Se deficient non-supplemented calves at 22 day after weaning (Beck et al., 2005). Also, placental transfer of Se is more efficient than milk transfer for calves from cows that received Se supplementation (Enjalbert et al., 1999).

Supplementation of Se can decrease infectious disease challenge and promote antibodies in response to antigen challenges. Titers of infectious bovine rhinotracheitis virus in steers increased from d 0 to 7 after the infectious challenge and decreased from day 7 to 21 in the Sel-Plex and selenite steers but continued to increase through 21 d after the challenge for non-supplemented steers (Covey et al., 2010). Also, it was reported that although supplementation of 1.7 mg Se/d had minimum effects on immune function of weaned beef steers (Fry et al., 2005), blood Se levels over 100 mg L^{-1} are needed to maintain optimum immunocompetence (Nicholson et al., 1993).

The effect of organic versus inorganic Se supplementation on bioavailability and immunocompetence in beef cattle has been initially evaluated. The supplementation of organic Se to diets of weaned beef steers increased Se content and

GSH-Px than inorganic Se (Fry et al., 2005). Supplementation of organic Se resulted in more available Se for calves than inorganic Se. Organic Se supplementation also resulted in higher immunocompetence than inorganic Se. Also, supplementation of Se-yeast of pre-weaning calves increased macrophage phagocytosis compared with sodium selenite (Beck et al., 2005). The ability of yearling beef cattle supplemented with Se to produce antibodies in response to antigen challenges with sheep red blood cells and ovalbumin tended to be higher for Se-enriched yeast than inorganic Se supplemented cattle (Nicholson et al., 1993). This greater effect by organic Se versus inorganic Se in ruminants could be a result of the rumen microbial reducing conditions converting inorganic Se into low available insoluble forms. However, without conversion, the organic Se can be used directly.

In summary, dietary Se supplementation in inorganic form is not adequate to meet high Se demand of growing, reproducing and lactating animals, and is less than adequate for further metabolism because of reduction to metallic Se or selenide by rumen bacteria, resulting in much lower retention level than organic Se. The organic Se such as selenized yeast in the form of Sel-Plex is better than inorganic Se due to increased Se concentration in blood and GSH-Px activity, approximately doubled Se concentration in colostrum and milk, higher Se transfer via placenta. As a result, cattle health is improved with lower somatic cell counts, decreased mastitis and retained placenta as well as improved conception rates. The benefit to the newborn calves is due to improvement of their antioxidant defenses and thermoregulation leading to better immunity, viability and lower mortality during first months of the postnatal development. It's also beneficial that organic Se SeMet can build Se reserves in their tissues, in particular in muscles and those reserves can be effectively used by cattle in

stress conditions when Se requirement is increasing while feed consumption is declining.

Effects of Se Supplementation on Hepatic Gene Expression

Selenium supplementation increased the expression of genes involved in antioxidant defense, lesion-protection, and apoptosis under the tumorigenesis. cDNA array showed that Se deficiency in rats led to a down-regulation of Se-dependent cGPx, significant down-regulation of genes important in the inhibition of apoptosis (defender against cell death 1 protein, Bcl2-L1), cell cycle (G1/S-specific cyclin D1) and antioxidant defense (gamma -glutamylcysteine synthetase catalytic subunit). Furthermore, an induction of genes encoding for detoxifying enzymes in liver (cytochrome P450 4B1, UDP-glucuronosyltransferase 1), and combined vitamin E and Se deficiency affects the expression level of genes encoding for proteins involved in inflammation (multispecific organic anion exporter, SPI-3 serine protease inhibitor) and acute phase response (alpha-1 acid glycoprotein, metallothionein 1) (Fischer et al., 2001). Dietary Se deficiency decreased mRNA levels of 7 common genes (Gpx1, Gpx4, Sepw1, Sepn1, Sepp1, Selo, and Selk) coding for selenoproteins involved in oxidation- and/or lesion-protective effect in liver of chicks (Huang et al., 2011). Se-supplemented diets also increased expression of SelW and selenocysteine-synthase in the liver of chickens compared to non-Se-supplemented chickens (Sun et al., 2011). Supplementation of Se for 2 weeks to rats reduced protein kinase c-alpha (PKC-alpha) gene overexpression in preneoplastic liver and the formation of preneoplastic lesions in the liver (Chen, 1993). Furthermore, 2.1 mg Se/kg Se-enriched broccoli diets for 10 weeks increases and ikBalphakappaB, hsp86, gadd45 gene transcripts and activates pro-apoptotic genes linked to p53, NFkappaB in response to "danger signals" such as tumorigenesis to the liver of rats compared to 0.11 mg selenium/kg control diet (Zeng

et al., 2003). The increased antioxidative capacity induced by those genes could explain the better immune-competence for Se supplemented beef cattle.

As for genes involved in iron metabolism, the expression of transferrin, transferrin receptor, and iron regulator protein1 mRNA were more abundant in Se-deficient (non-supplemented Se) than in Se-adequate (0.15 mg Se/kg as sodium selenite for 15 wk) liver (Christensen et al., 2000). In addition, supplementation of Zn and Se can protect against Cd-induced toxicity by alteration of metallothionein gene expression in the liver of rats (Banni et al., 2010).

The mRNA levels for transthyretin and citrate transport proteins are both reduced in the livers of Se-deficient rats compared to Se supplemented rats with 0.5 mg Se/kg diet for 13 weeks (Kendall and Christensen 1997). The solute carrier gene Slc48a1 is up-regulated in the liver of rats with selenium-deficient diets compared to selenium-supplemented diets (Mallonee et al., 2011).

Other genes found to be differentially expressed upon Se supplementation compared to non-Se supplementation are involved in other metabolic pathways. The mRNA levels for liver estrogen sulfotransferase isoform-6 alpha 2u-globulin were markedly reduced in Se deficiency compared to rats supplemented Se with 0.5 mg Se/kg diet as sodium selenite to the rats for 13 weeks (Yang and Christensen 1998). Furthermore, 50 g/kg Se supplementation for 60 days down-regulated the expression of phospholipase D mRNA in the liver of diabetic rats compared to non-supplemented rats (Wu et al., 2006).

Organic and inorganic Se supplementation also differentially affects the gene expression of liver. Genes in the liver of mice related to the selenoproteins Gpx1, Selh, Sep15, and Sepw1, were differentially expressed among Se supplemented diets (sodium selenite, SeMet, or a yeast-derived Se) (Mallonee et al., 2011). Also, in mice,

non-selenoproteins encoded by Se-responsive genes including transport and stress response are differentially induced by three Se supplemented diets (Mallonee et al., 2011). Because most of selenoproteins function as peroxidases, the different expression of genes related to selenoproteins, and some genes related stress response, upon organic versus inorganic Se supplements could explain the different antioxidative capacity of beef cattle supplemented with organic forms of Se.

In summary, Se supplementation has different effects on the expression of genes in the liver compared to non-supplements. Differentially expressed genes are involved in 1) oxidation/lesion, apoptosis; 2) iron metabolism; 3) carrier proteins and transporters; 4) other metabolisms.

Chapter 3: Hepatic transcriptome profiles differ among maturing beef heifers supplemented with inorganic, organic, or mixed (50% Inorganic:50% Organic) forms of dietary selenium

Introduction

Selenium is a metalloid belonging to Group VIA of the Periodic Table, the same as sulfur. Selenium plays an important role in regulation of various physiological functions in beef cattle including immunity, reproduction and early postnatal viability (Suzuki, 2005). Selenium deficiency alone, or in combination with vitamin E, is associated with reduced growth and productivity rates, early mortality and immune-suppression. Se deficiency has been specifically linked with the development of various diseases in ruminants (Pehrson, 1993; Kolb and Seehawer, 2001) including nutritional muscular dystrophy (white muscle disease, the most well documented disease related to Se/vitamin E deficiency in ruminants), retained placenta and metritis (affecting about 9% of USA dairy industry births), and mastitis (estimated annual cost is \$17,500 for a 100-cow herd) (Surai, 2006).

The concentration and availability of Se in the soil primarily determines the Se content of forages. The Se requirement for beef cattle among all ages is 0.10 mg Se/kg of diet daily (NRC, 2000). The Se content in forages (and grains) of the southeast USA (including Kentucky) is low (< 0.05 mg Se/kg) to variable (< 0.1 mg/kg) (Ammermann et al., 1975). Consistently, a survey of whole blood Se concentrations of beef cows and heifers from 253 cow-calf operations in 18 states found that 42% of southeastern USA (including Kentucky) cattle were Se deficient ($\leq 0.080 \mu\text{g/mL}$), as opposed to 18% nationally (Dargatz and Ross, 1996). Therefore, Se supplementation of forage-based diets is necessary in Kentucky.

Supplementation of diets with Se is regulated by the Food and Drug Administration and cannot exceed 120 mg Se/kg, or an intake of 3 mg per day, when

delivered in free-choice mineral supplements to cattle (FDA, 1987). Diets can be supplemented with inorganic or organic forms of Se. Sodium selenite and sodium selenate are the typical forms used, despite the knowledge that Se primarily is present in plants as organic forms. Organic forms of Se include selenomethionine (SeMet) and selenocysteine (SeCys). However, commercial animal diets are not directly supplemented with SeMet or SeCys. Instead, SeCys and SeMet are supplied as constituents of Se-enriched yeast extracts (e.g., SeMet is the predominant form of Se in Sel-Plex (Alltech Biotechnologies, Inc., Nicholasville, KY) (Korhola et al., 1986).

The effect of supplementing diets with inorganic versus organic Se supplements on Se bioavailability (concentrations of Se in blood and tissues) and bioactivity (blood glutathione peroxidase activity) have been compared. For example, assimilation of Se after 105-106 days by whole blood, red blood cells, and biopsied liver tissue was greater for slow maturing beef heifers fed 3 mg/day of Se as Sel-Plex than for sodium selenite (Liao et al. 2011). Similarly, Se concentrations ($\mu\text{g/g}$) in the skeletal muscle of cows supplemented with 3 mg/day as Se-yeast was 1.5-2.5 fold higher than when supplemented with sodium selenite (Ortman and Pehrson, 1999).

With regard to the effect of organic versus inorganic Se on animal performance under stress conditions, supplementation of Se in organic forms is known to decrease the mean milk somatic cell counts more than inorganic forms in experimental (McIntosh et al., 2002) and commercial (Harrison et al., 2005) cow herds. However, supplementing cattle diets with organic versus inorganic forms of Se did not affect the average daily gain of heifers supplemented with 3 mg Se/day as sodium selenite or Sel-Plex[®] for 105-106 days (Liao et al., 2011). Likewise, there was no difference on the final body weight, average daily gain, or gain-to-feed ratios of

slow maturing beef heifers supplemented with 3 mg Se/day as sodium selenite, Sel-Plex, or a 1:1 mix of sodium selenite and Sel-Plex for 224 days (Brennan et al., 2011).

To better understand effect of supplementing beef cattle diets with different forms of Se on hepatic function and hepatic Se-specific metabolism, so that appropriate dietary Se supplementation regimens for beef cattle can be developed, it would be useful to understand if hepatic gene expression profiles differed in beef cattle fed the same diet but containing different forms of Se supplement. Achieved differences in small RNA (mRNA, miRNA) profiles would be indicative of altered physiological capacities. Our previous study examining the effects of organic versus inorganic 3 mg Se/day supplementation for 105 - 106 days on hepatic gene expression profile of beef cattle (Liao et al., 2011) revealed that organic and inorganic Se supplements commonly and differentially affected the hepatic gene expression compared to non-supplemented animals. These Se form-induced differences corresponded with 18 and 59% increases in hepatic content of Se by the inorganic and organic Se supplements. However, because only a single sample point (day 105/106) was used, it was not known if stabilization of hepatic Se assimilation had been achieved. Thus, conclusions about whether observed Se form induced difference in liver gene expression profiles represented stable or transient changes could not be drawn.

To address this issue, and to determine the effect of a mix of inorganic and organic forms (Mix) consisting of 1.5 mg Se/day as sodium selenite and 1.5 mg Se/day as Sel-Plex[®], a second experiment using the same animal model (maturing beef heifers gaining 0.5 kg/day on a cottonseed hull-based diet) was conducted over a 224-day period using (n = 10) none (Control) or 3 mg Se/day dietary supplement treatments as sodium selenite (ISe), Sel-Plex (OSe), or Mix to determine the effect of

Se supplement form on tissue assimilation of Se (Brennan et al., 2011). Se assimilation ($\mu\text{g/g}$) by liver tissue of ISe, OSe, and Mix treatment heifers was maximal and stable from d 56 through d 224 for all treatment groups, but the amount of Se assimilated by liver tissue was dependent on the Se treatment (OSe = Mix > ISe > non-supplemented Control heifers).

Using the liver tissue collected from these (Brennan et al., 2011) maturing beef heifers that had assimilated Se supplement form-specific amounts of Se, the objectives of the current work were to (1) compare hepatic gene transcriptome profiles when hepatic liver assimilation was stable (d 168) using microarray methods, and (2) conduct bioinformatics analysis of these profiles to predict altered hepatic physiological capacities induced by specific forms of Se supplement.

MATERIALS AND METHODS

Source of Liver Tissue, Experimental Treatments, and Liver Se Concentrations

The liver tissue samples used were from the animals of the Brennan et al. (2011) trial, for which all animal procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee. Specific descriptions of the animal model (Brennan et al., 2011) and liver biopsy procedures used (Brown et al., 2009; Brennan et al., 2011) have been described. However, briefly, at the end of a common Se depletion period and diet-adaptation period, heifers were ranked (in groups of four) on the Se concentration of their biopsied liver sample. Within a rank, heifers were randomly assigned ($n=10/\text{treatment}$) to one of four dietary Se supplementation treatment groups, to stratify similar basal liver Se content among treatment groups. For the control treatment (Control) no exogenous source of Se was supplied to the basal mineral-vitamin premix. For the ISe, OSe, and a 1:1 combination of ISe and OSe (Mix), the basal mineral-vitamin premix was supplemented with sodium selenite

(ISe, Prince Se Concentrate; Prince Agri Products, Inc., Quincy, IL, USA), Se-enriched yeast (OSe, Sel-Plex[®], Alltech Inc., Nicholasville, KY, USA), or their combination, respectively. The Control, ISe, Mix, and OSe mineral-vitamin pre-mixes contained 1.09 mg/kg, 36.1 mg/kg, 34.74 mg/kg and 34.56 mg/kg of Se, respectively. Animals were weighed biweekly and intake of the basal diet adjusted, to ensure animals maintained an average daily gain of 0.5 kg/day. The daily supply of Se for each of the ISe, Mix, and OSe treatment rations was calculated to provide 3.0 mg/day.

After biopsy, the liver sample from each animal was placed in foil packs, snap-frozen in liquid nitrogen, and stored at -80 °C until analyzed for Se concentration or RNA extraction. The liver Se concentrations were analyzed as previously reported (Brennan et al., 2011) by Donald C. Mahan (The Ohio State University, Columbus, OH, USA) and differences in Se-form treatments reported (Brennan et al., 2011). Specifically, Se concentrations in liver were greater ($P \leq 0.01$) in all three Se-supplementation treatment groups than the non-supplemented Control group, whereas liver Se concentrations of Mix heifers were greater ($P = 0.01$) than for ISe heifers, OSe heifers tended ($P = 0.08$) to be greater than ISe heifers, and Mix heifer liver Se concentrations did not differ ($P = 0.20$) from OSe heifers (Brennan et al., 2011).

RNA Extraction and Analysis

For each animal, total RNA was extracted from 400 mg of frozen liver tissue using TRIzol Reagent (Invitrogen Corporation, Carlsbad, CA) following the manufacturer's instructions. The purity and concentration of total RNA samples was analyzed by a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE), which revealed that all samples were of high purity with 260/280 absorbance ratios of 2.0-2.1 and 260/230 absorbance ratios ranging from 1.5 to 1.9. The integrity of total RNA was examined by gel electrophoresis using Agilent 2100

Bioanalyzer System (Agilent Technologies, Santa Clara, CA) at the University of Kentucky Microarray Core Facility. Visualization of gel images and electropherograms showed that all RNA samples had high quality with RNA integrity numbers (RIN) being greater than 8.8 and 28S/18S rRNA absorbance ratios greater than 1.8.

Microarray Analysis

The custom WT Btau 4.0 Array (version 1) (GeneChip; Affymetrix, Inc., Santa Clara, CA), which contains 26,303 bovine gene transcripts, was used. Microarray analysis was conducted according to manufacturer's standard protocol at the University of Kentucky Microarray Core Facility. Briefly, 3 µg RNA for each sample was reverse-transcribed to cDNA first, and then from cDNA (double-stranded) to cRNA (single-stranded) which were then labeled with biotin. The biotinylated cRNA were further fragmented and used as probes to hybridize the GeneChips in the GeneChip Hybridization Oven 640, using 1 chip per RNA sample. Due to the loss of one animal, and failure to obtain one liver sample, the experimental observations for each treatment were as follows: n = 9 (one missed sample), 9 (one animal died), 10, and 10 for Control, ISe, Mix, and OSe treatments, respectively.

The raw expression intensity values from the 38*.cel files from the raw methylation measurements were imported into Partek Genomics Suite software (PGS, version 6.66 beta; Partek Inc., St. Louis, MO). For GeneChip background correction, the algorithm of Robust Multichip Averaging adjusted with probe length and GC oligo contents was implemented (Irizarry et al., 2003; Wu et al., 2004). The background-corrected data were further converted into expression values using quantile normalization across all the GeneChips and Median Polish summarization of multiple probes for each probe set (Partek, 2009). The similarity matrix was generated

by Pearson (Linear) Correlation and was converted to heat map to visualize the correlation among chips. One Mix and one OSe treatment chip were identified as outliers because of their relatively low correlation with any of other GeneChips. The average correlation between any pair of GeneChips 38 was 0.975 except for two of the chips (one belonged to Mix, and one to OSe) that had correlations of about 0.943 and 0.837 with the other chips (Figure 1a). These two chips were removed prior to further statistical analysis. New correlation heat map (Figure 1b) after removing outliers showed no outliers, the final correlation meets the 0.984. The remaining 36 *.cel files (n=9 for each of the Se treatments) were imported into PGS and subjected to the background correction and normalization as decided above.

GeneChip transcripts were annotated using the NetAffx annotation database for Gene Expression on Bovine GeneChip Array, provided by the manufacture and last updated September 2011. When appropriate, incompletely annotated transcripts were subjected to GenBank analysis and annotation updated. Quality control of the microarray hybridization and data presentation was performed by MA-Plot on all gene expression values and Box-Plot on the control probe sets on Affymetrix GeneChips (data not shown).

Principle component analysis (PCA) was conducted to determine the quality of the microarray hybridization and visualize the general data variation among the chips (Partek, 2009). Differentially expressed genes (DEG) were subjected to hierarchical clustering (Partek, 2009) and Ingenuity Pathway Analysis (IPA, 8.7-3203; <http://www.ingenuity.com>; Ingenuity Systems, Inc., Redwood City, CA).

MicroRNA analysis

Selected microRNA (miRNA) were imported into the miRNA database (<http://www.mirbase.org/index.shtml>) to search and browse their hairpin precursor and mature sequences, and their annotation, including accession or ID or pubmed ID. To predict their targets, miRNA sequences were imported into the TargetScan (<http://www.targetscan.org>). The biological targets of *Bos taurus* miRNA were identified by searching for the presence of (1) conserved branch length sites that matched the seed region of each miRNA (Lewis et al., 2005), (2) sites with mismatches in the seed region that are compensated by conserved 3' pairing (Friedman et al., 2009), (3) and poorly conserved sites. Putative miRNA targets were compared to DEG found to be affected ($P < 0.01$) by Se treatment. The DEG that were putative targets of miRNA were subjected to the miRNA Target Filter analysis component of IPA.

Statistical Analyses

Animal was the experiment unit and one GeneChip per animal was used to evaluate relative hepatic mRNA content in response to Se treatment. Se treatment effects on expression of all 26,303 array transcripts were determined by ANOVA. Source of variation analysis determined the sources of variation associated with treatment and error by calculating the mean F ratio of all 26,303 transcripts by Partek Genomic Suite (6.66 beta version) software. Gene transcripts were considered differentially expressed at $P < 0.01$. Treatment least square means of DEG were separated using the pdiff option of SAS (9.2 version, SAS Inst., Inc., Cary, NC, USA).

RESULTS

Principle Component Analysis (PCA)

The mean F ratios for treatment and error were 0.90 and 1.00, respectively (data not shown). Principle component analysis revealed a total variance of 16.7% (Figure 2). The principal component (PC #1, x-axis) contained transcripts with a higher degree of variance 9.08%, whereas PC #2 (y-axis) encompassed genes that had lower 7.58% ranges of variance. In general, the GeneChips within each treatment group were clustered together and the Control, OSe, and Mix treatment groups were clustered closer than the ISe.

Se Treatment-specific Profiles of Differentially Expressed Genes (DEG)

Analysis of variance analysis found that the expression of 139 transcripts were differentially affected ($P < 0.01$) by Se supplementation (Table 1). Of the 139 DEG, the nucleotide or protein sequences have been validated. The range for Se treatment induced expression of annotated genes ranged from 2.67-fold upregulated (BOLA-DQA20) to 1.65-fold downregulated (MIR2393). Among these 139 DEG some were uniquely and some commonly affected by Se treatment (Figure 3). Relative to expression by Control heifers, 32 genes were solely affected by ISe treatment, with another 22 and 4 being commonly affected by ISE and Mix or OSe treatments, respectively. In contrast, OSe supplementation solely affected the expression of 22 genes and commonly the expression 4 and 7 genes with ISe and Mix, respectively. For the Mix treatment, 33 genes were solely affected, but another 22 and 7 were commonly expressed with ISE and OSe, respectively. In addition, 11 genes were affected commonly by all three Se supplementation treatments relative to Control, whereas the expression of another 8 genes were differentially expressed among ISe, Mix, and OSe treatments, but did not differ from Control expression levels.

Bioinformatic Analysis of Se Treatment-specific Profiles

In total, 69 (ISe), 73 (Mix), and 44 (OSe) DEG were solely or commonly affected by Se supplementation treatments. The annotated genes within a Se treatment were subjected to bioinformatic analysis to gain insight into differences in the physiological capacities that might result from the separate Se treatments. Network analysis revealed several common and solely-affected networks. The common network identified for ISe (ACIN1, GCG, GCLC, GHRH, ITGA2, Mir-222, MMP9, NPY1R, PDX1) and Mix (ACIN1, CCNB2, CDH4, CXCL2, FCGR2A, GCLC, ITGA2, MMP9) DEG was cellular movement. The common network identified for OSe (CNGA1, GCLC, ITGA2, LDHB, LRRTM2, PLCZ1, OPTC, RIN2, RUFY3, SEPW1, TRPC5, VAMP5, ZNF238) and Mix (EIF4A1, FCGR2A, LALBA, PLCZ1, OPCT, SEPW1, SHD, TNFAIP8L2, TRPC5, TULP3) was cell-to-cell signaling and interaction.

No networks were identified solely for OSe treatment. In contrast, the solely identified networks for ISe were cellular growth and proliferation, DNA replication, recombination, and repair, cellular movement (CAPRIN2, CEND1, CNGA1, LRRTM2, NTM, SEPW1, SHD, THOC5, ZNF703) and organ development, carbohydrate metabolism, tissue development (CDH4, CEP350, GCM1, LALBA, Mir-222, OPCT, SNAPIN, TRPC5, THG1L). For the Mix treatment, solely identified networks were cardiac proliferation, cardiovascular system development and function, cellular growth and proliferation (CEP350, CNGA1, FRAS1, GOLGA3, HBE1, IRX4, LEPRE1, MTMR2, PSME4, SNAPIN).

Hierarchical Clustering of GeneChips into Se Treatment-specific Groups

Hierarchical cluster analysis of the 139 DEG (Figure 4) revealed four distinct GeneChip clusters (9 GeneChips each) that were segregated by Se treatment and that were consistent with the PCA results. Specifically, OSe treatment GeneChips were

more closely aligned with Control GeneChips than were Mix GeneChips, and the ISe cluster was similar to Mix GeneChips and most dis-similar to Control GeneChips.

Bioinformatic Analysis of Clustered DEG

Hierarchical clustering of the 139 DEG also identified 8 patterns of gene expression that were sensitive to Se-treatment (Figure 4). To identify potential metabolic relationships among DEG within a cluster in Table 1, bioinformatics analysis was conducted on annotated DEG within a cluster. The predominant (the highest ratio of DEG to all molecules) networks were identified: Cluster 1, organismal disease (SEPW1) and molecular and mineral transport (TRPC5); Cluster 2, cell to cell signaling and interaction (PCDHB14, RUFY3); Cluster 3, tissue development and protein synthesis (CCNB2, CXCL2, DDHD1, GCG, MTMR2, PSME4, SNHG3-RCC1, RIN2); Cluster 4, cell cycle (GSG2) and tissue development (QPCT, MFI2); Cluster 5, cell movement development (CDH4, CEND1, CNGA1, ITGA2, LALBA, MMP9, NTM, PCDHA2, SHD, ABO) and interaction (FGF22); Cluster 6, regulation of hormone (GHRH, Mir-222, PDX1) and skeletal disorders (FBXL13); Cluster 7, cellular function (LDHB, LRRTM2, PLCZ2, TNFAIP8L2, VAMP5, FRAS1) and developmental disorder (FRAS1); Cluster 8, cell signaling, molecular and mineral transport, and metabolism (ACIN1, FCGR2A, GOLGA3, LEPRE1, NPY1R, SNAPIN, SRP72, ZNF236, THG1L, THOC5) cell growth and tissue function (THG1L, ZNF22) DNA replication repair (ANDP2).

Differentially Expressed MicroRNA (MiRNA)

Six hundred twenty-one miRNA are detectable by the bovine GeneChip used for this study. Of these, 621 were expressed, but only three MiRNA (MiR-222, Mir-2393, Mir-2300b) were identified among the 139 DEG (Table 1). Among the remaining annotated DEG, 1 putative mRNA target was identified for Mir-222, 20 for

Mir-2393, and 9 for Mir-2300b (Table 2). Among these mRNA targets, DDHD1 is common to both Mir-222 and Mir-2393, whereas RUFY3, LRRTM2, FRAS1 are common targets of both Mir-2393 and Mir-2300b.

Bioinformatics analysis of the mRNA targets of each differentially expressed MiR was conducted to identify potential physiological consequences of Se treatment alteration of MiR expression (Table 2). Whereas MiR-222 is predicted to affect only one network, the 17 of the 20 differentially-expressed targets of MiR-2393 are associated with 6 networks, the most prominent (9 mRNA) being Cell Cycle, Cancer, and Neurological Disease. For MiR2300b, 7 of 9 putative target mRNA are identified with 4 networks, the most prominent (4 mRNA) being Cell Cycle, Cellular Assembly and Organization, DNA Repair Replication, Recombination, and Repair.

DISCUSSION

Experimental Model

Little is known regarding the relationship between Se concentrations in the liver and liver gene expression, especially in cattle. The effect of Se supplementation on liver gene expression using a Se-deficient versus Se-supplemented experimental model in rats (Fischer et al., 2001) and chicks (Huang et al., 2011; Sun et al., 2011) have been reported. However, because most cow-calf operations in regions of Se-poor soils provide enough Se in diets to avoid Se deficiency. the goal of this research was to determine the effects of three Se supplementation strategies (ISe, Mix, OSe) on liver gene expression profiles using a Se-adequate experimental model.

Descriptions of the experimental model, diets, and liver tissue collection, and Se content in the liver samples used for this trial have been published (Brennan et al., 2011). However, briefly, liver Se content ($\mu\text{g/g}$) for Control (0.08 mg Se/day) group was essentially stable throughout the trial, whereas the Se concentration in ISe, OSe,

and Mix heifers increased to about 56 to 112 days and then were stable for the remainder of the trial (Figure 5, Brennan et al., 2011). The total daily Se consumption by individual heifers of the Control, ISe, Mix, and OSe treatment groups was 0.82, 3.80, 3.69, and 3.76 mg/day, respectively. The liver Se concentration ($\mu\text{g/g}$) of these heifers at day 168 were 0.24 (Control), 0.40 (ISe), 0.49 (Mix), and 0.49 (OSe) and differed (OSe = Mix > ISe > Control) (Brennan et al., 2011). For the Control, ISe, and OSe treatments, these Se concentrations levels are consistent with those found in the previous pilot trial (Liao et al., 2010), which had liver Se concentrations ($\mu\text{g/g}$) of 0.26, 0.34, and 0.47, respectively, from maturing Angus-cross heifers consuming 0.48, 3.48, and 3.48 mg Se/day for 105 – 106 days (Liao et al., 2010). Importantly, Se concentrations of 0.25 to 0.50 $\mu\text{g/g}$ in liver tissue are considered normal (Ullrey, 1987; Corah, 1996; Surei, 2006). Thus, the animal model used for both the present (and pilot) trial is robust with respect to both Se-adequacy and Se form-dependent Se assimilation by liver tissue.

Se Assimilation Levels and Gene Expression Profiles

As just described, Se treatments affected liver Se assimilation by heifers.

Microarray analysis was used to determine the effect of Se treatment on gene expression (relative mRNA and miRNA levels) and, by extension, Se treatment-induced differences in physiological capacities. Microarray analysis revealed clear differences in the hepatic gene expression profiles of the four Se treatment groups (Figure 4). However, inter-treatment differences in Se content did not match differences in gene expression profiles. That is, although ISe-induced hepatic Se concentrations were more similar to Control concentrations, ISe-induced hepatic gene expression profiles were most different from Control profiles. Likewise, although the hepatic content of Se in Mix and OSe treatments were essentially identical, OSe

expression profiles were more similar to Control, and Mix to ISe (Figure 4). Thus, absolute hepatic Se assimilation was not a good indicator of gene expression. Instead, the form of Se consumed may be a better indicator. That is, the pattern of hepatic DEG was most similar between the treatments with the greatest proportion of organic Se (Control, lowest hepatic Se content) and OSe (one of the highest Se content), while the hepatic DEG expression profiles were similar between those animals consuming proportionately increasing amounts of inorganic sources of Se (Mix and ISe). Therefore, it appears that the form of Se consumed may be more important than the amount of Se assimilated. Based on this conclusion, future trials should assess the relationship between total Se assimilation, the form of Se assimilated, and patterns of gene expression.

Common and Differential Selenium Form-Induced Metabolic Pathways

Because the metabolic fates and pathways for OSe (SeCys, SeMet) and ISe (selenite) have distinctive, as well as common, components (Figure 6), an unexpected finding of this study was that the expression of only a few of the genes encoding proteins responsible for Se-specific metabolism were altered. Although expression of mRNA for 16 of 20 of Figure 6 proteins were detected by the GeneChips (data not shown), none of these were altered by Se-supplemental treatment. Assuming that Se might be limiting in non-supplemental heifers, these findings are somewhat unexpected given that supplementation of organic Se should result in proportionately greater amounts of SeCys to incorporate into the “regulated” selenoprotein synthesis pathway (tRNA_{Sec}-mediated incorporation of SeCys into “selenoprotein” polypeptide chains), the “unregulated” pathway (non-specific competition between Met and SeMet binding by tRNA_{Met} and incorporation into “Se-containing proteins”), and the trans-selenation pathway (Figure 6, Suzuki, 2005). In contrast, with the exception of

minor amounts of organic Se compounds contained in Se-assimilating ruminant microbes, inorganic dietary Se will not be metabolized through the the regulated pathway and its associated recycling capacity. However, the findings from this study indicate that the non-supplemental Control treatment expressed adequate amounts of these proteins and, thus, supports the understanding that our animal model (slow-growing, maturing beef heifer) is one of Se adequacy, not deficiency.

Twenty-three of the 25 known mammalian selenoproteins (all but SEP15 and SEPN) were assayed by the microarray. Of these, only expression of SEPW1 was altered (Table 1). These results indicate that precursors and enzymes responsible for SeCys formation and incorporation into polypeptide chains were not lacking in non-supplemented Control heifers. The upregulation of SEPW1 mRNA expression is consistent with this genes' known sensitivity to supplemental Se. In this regard, the upregulation of SEPW1 mRNA indicates that the model was capable of responding to dietary Se supplementation.

Functional Analysis of Heiarchical Clusters

The hierarchical clustering of 139 DEG identified 8 patterns of gene expression that were sensitive to supplemental Se-treatment (Figure 4). Functional analysis was performed on the annotated DEG within each cluster to gain insight into the potential physiological significance of these responses. Eight transcripts from Cluster 1 are involved in cation transport and antioxidative activity. SEPW1 (selenoprotein W-1) contains SeCys, encoded by the UGA codon. SEPW1 belongs to the selenoprotein W subfamily, of the selenoprotein WTH family, and functions as an antioxidant enzyme. Specifically, SEPW1 binds to glutathione which targets reactive oxygen species such as hydrogen peroxide, superoxide anion radical, and hydroxyl radical, which are toxic by-products of cellular oxygen metabolism (Jeong et al.,

2002). The binding sites are thought to be SeCys¹³ and Cys³⁷ residues (Jeong et al., 2002). Homologues of transient receptor potential (TRP) genes encode a variety of cation channels, most of which conduct Ca²⁺ across the plasma membrane (Mori et al., 2011).

Transient receptor potential (TRP) channels play pivotal roles in sensing and adapting to a wide variety of environmental changes (Yamamoto et al., 2010). Immunolocalization studies have revealed that TRPC5 is distributed on both the apical and basal membrane in the endothelial cell layer of vascular tissue. TRPC5 channels are activated by nitric oxide and inflammatory mediators via oxidative (S-nitrosylation) modification of cysteine residues, which triggers the Ca²⁺ influx and other adaptation reactions after sensing the oxidative stress. This study found that the TRPC5 expression is equally stimulated by all forms of Se supplement. Thus, if mRNA levels represent functional capacity, then Se supplementation induced an increased capacity to respond to oxidative stress in Se-supplemented heifers versus the Se-adequate, but non-supplemented Control heifers.

The cell-to-cell signaling was one pathway that indentified in Cluster 2 DEG. PCDHB14 expression was increased 1.28- to 1.35-fold by ISe and Mix treatments, respectively. In contrast, CYP2D14 and RUY3 expression were decreased by OSe. PCDHB14 is a cadherin protein that belongs to the protocadherin family. As such, PCDHB14 likely is involved in the modulation of synaptic transmission and the generation of specific synaptic connections (Frank and Kemler 2002). The up-regulation of PCDHB14 by Mix and OSe Se treatments indicates a more active synaptic signal transmission.

Cluster 3 DEG are involved in tissue development and protein synthesis. The Mix treatment up-regulated 13 of 15 genes in this cluster, whereas ISE downregulated

the gene for glucagon (GCG) and OSe downregulated RIN2. DDHD1 can generate the arachidonic acid-containing lysophosphatidylinositol that increases both the phosphorylation of the extracellular signal-regulated kinase (ERK) and intracellular Ca^{2+} levels (Yamashita et al., 2010). SNHG3-RCC1 is the regulator of chromatin condensation 1 that was the guanine nucleotide-exchange factor for the Ran GRPase, and functions as the nucleo-cytoplasmic transport, mitosis, and nuclear-envelope assembly. RCC1 has a propeller-like structure, one side binds to Ran, and the other side binds chromatin. Upon some post-translation modification, SNHG3- RCC1 can generate the RanGTP that is required for spindle assembly and chromosome segregation during the mitosis (Kline-Smith and Walczak, 2004).

The pattern for Cluster 4 DEG sensitivity to Se treatments was down-regulation (-1.16- to -1.65-fold), with the exception of MF12, which was upregulated (1.07-fold) by ISe treatment. GSG2 was down-regulated 1.23-fold by OSe treatment. GSG2 is involved in the chromatin modification especially the histone phosphorylation, which provides a chromatin binding site for chromosomal passenger complex at centromeres to regulate chromosome segregation during the mitosis (Wang et al., 2011). ZNF238 (Zinc Finger 238; RP58) also is a DNA binding protein and acts to repress transcription, likely by binding the Dnmt3a DNA methyltransferase. ZNF238 is a member of the POZ-zinc finger family (Okado et al., 2009), which are important regulators of DNA damage responses, cell-cycle progression, and many developmental events (Kelly and Daniel, 2006). Of the Cluster 4 DEG, MiR2393 was the most affected, being significantly downregulated 1.41- to 1.65-fold by Mix and OSE, and quantitatively down-regulated by ISe, respectively. As with ZNF238, MiR2393 acts to repress expression but by binding mRNA rather than DNA (see below).

Similar to MiR2392, QPCT (glutaminyl-peptide cyclotransferase or glutaminyl cyclase) mRNA expression also was affected by all 3 Se treatments, being downregulated by 1.16-, 1.26-, and 1.30-fold by ISe, Mix, and OSe, respectively. QPCT is involved in the synthesis of pyroglutamyl peptides by posttranslational cleavage of nascent gene products to their respective N-terminal pyroglutamyl bioactive peptides, including thyroid-releasing hormone, gonadotrophin-releasing hormone, and corticotrophin-releasing hormone (Fischer and Spiess, 1987). However, paradoxically, QPCT mRNA was not detected by a previous Northern blot analysis (Pohl et al., 1991), although strong expression in pituitary and most brain regions, and relatively, lesser expression by retina, kidney, thymus, and skeletal muscle. Thus, either our microarray detection of bovine QPCT mRNA expression by hepatocytes was more sensitive than Northern blot analysis, or differences in liver tissue samples assayed existed (slaughterhouse tissue versus animals of known origin). If our liver expression of QPCT is validated, then an important finding from this study is that Se supplementation may alter QPCT-mediated metabolism, most auspiciously (perhaps) that occurring in the pituitary.

Cluster 5 DEG genes were up-regulated by any form of supplemental Se relative to Control. The predominant network of Cluster 5 was cell movement development. CDH4 is a calcium-dependent cell-cell adhesion glycoprotein, that is comprised of cadherin repeats and that is important for tissue development. The CNGA1 encoded protein in plasma membrane increases translocation of Ca^{2+} and Na^{+} in extracellular space to Ca^{2+} in cytoplasm (Kaupp and Seifert, 2002). THOC5 78 kd FMIP protein decreases differentiation of macrophages (Tamura et al., 1999) and it is involved in the differentiation of monocytes (Carney et al., 2009) and can increase the differentiation of granulocytes.

ITGA2 encodes the alpha-chain of integrins found in T cells, fibroblasts, and is involved with cell adhesion and cell-surface mediated signaling. Importantly, the integrin complex can mediate the activation of hepatic stellate cells (Friedman, 2000). ITGA2 upregulation indicates greater capacity for healing and IL-3 mediated immune response. ITGA2 also is involved in activation of monocyte upon to trigger monocyte migration to inflammatory sites to regulate IL-13-mediated monocyte activation (Yakubenko et al., 2011). The expression of ITGA2 mRNA was upregulated equally (1.22- to 1.34-fold) by all 3 Se treatments. The upregulation of it for Se treatment indicates better tissue wound healing capacity in response to injury, and also indicates an increased activation of macrophages.

Although not identified as part of the cell movement development network by IPA analysis, GJB4 is a transmembrane connexin protein that forms gap junctions. Gap junctions provide for a unique system of intercellular communication allowing rapid transport of small molecules from cell to cell (Cancelas et al., 2000). GJB4 is expressed by the liver cobblestone area-forming cells and is responsible for the communication and supportive ability and GJB4-dependent gap junction can contribute to the regulation of the clonal outgrowth of hematopoietic progenitors (Cancelas et al., 2000). It also reported that GJB4 deficiency impairs hemopoiesis and the GJB4 knockout fetuses have a lower content of progenitor and stem cells in their liver as compared with their wt littermates. Besides, the GJB4 may participate in the function of B and T lymphocytes and macrophages thus influence the body immune function (Alves et al., 1996; Alves et al., 1998). GJB4 mRNA expression was upregulated 1.13- and 1.26-fold by ISe and Mix treatments, respectively, and tended to be upregulated (1.10-fold) by OSe treatment. This upregulation suggests that

heifers consuming inorganic and mixed forms of Se had greater capacities for hepatic GJB4-mediated cell-to-cell communication and immune system function.

SLC6A17 is a glutamatergic- and GABA- (somewhat) specific neuron transporter responsible for Na⁺-dependent absorption of (primarily) glutamine and proline from the cytosol into synaptic vesicles (Zaia and Reimer, 2009). In liver tissue, SLC6A17 was equally upregulated by ISe and Mix. If the bovine expression pattern for SLC6A17 is the same for other species, then this finding indicates the the glutamatergic neurons which innervate the liver have an increased neural propagation function, due to the increased capacity to absorb the precursor for glutamate, glutamine.

For Cluster 6 DEG, ISe treatment increased gene expression for 8 of 11 annotated DEG, whereas Mix decreased expression of 3 genes and OSe had no effect. The predominant network found for Cluster 6 genes was regulation of hormone metabolism (GHRH, PDX1). GHRH is known to stimulate NF-kappa beta transcription factor that activates angiogenic factor and expression of genes involved in encoding enzymes in the prostaglandin-synthesis pathway (NCBI, <http://www.ncbi.nlm.nih.gov>). The transcriptional factor pancreatic duodenal homeobox 1 (PDX1) plays a pivotal role in pancreatic beta-cell differentiation, and insulin gene expression and synthesis (German et al., 1995; Offield et al., 1996; Ahlgren et al., 1998; Bernal-Mizrachi, et al., 2001). We found PDX1 mRNA was upregulated 1.17-fold by ISe but not affected by Mix or OSE treatments. Although it has been demonstrated that PDX expression can be increased through hyperacetylation of H3 and H4 histones of the Pdx1 promoter in response to overexpression of Se-dependent cellular glutathione peroxidase-1 (GPX1) (Wang et al., 2008), GPX1 expression was not affected by Se treatment (data not shown).

Therefore, whether PDX1 expression was induced directly by increased inorganic concentrations due to ISe treatment effects, indirectly through post-transcriptional effects on GPX1, or by a different mechanism, remains to be determined.

Of the 10 DEG in Cluster 7, 2 (LDHB, VAMP5), were upregulated by OSe treatment whereas 6 were down regulated by ISe treatment, 1 by ISe or Mix, and 1 by Mix alone. Genes in Cluster 7 are involved in the membrane adhesion (FRAS1), immune function (TNFAIP8L2), and glycolysis (LDHB) networks.

The effect of Se treatment on Cluster 8 genes was similar to that of Cluster 7 genes; OSe increased expression of 5 of 20 genes in Cluster 8, whereas ISe (5), ISe and Mix (6), or Mix alone (4) decreased expression of DEG. These DEG are involved in cell signaling, molecular and mineral transport, and tissue growth and function.

Cluster 8 also contained two of the most strongly affected DEG in the trial. *Bos taurus* major histocompatibility complex, class II, DQ alpha 2 (BOLA-DQA2; Morooka et al., 1995) BOLA-DQA2 mRNA expression was increased 2.67-fold by OSe, but was not affected by ISe or Mix treatments. This finding suggests that OSe-consuming animals may have a greater capacity to respond to pathogen challenges (Handunnetthi et al., 2009; Hou et al., 2012) assuming that OSe heifers were not stimulated by a pathogen challenge not present to the other treatment groups. The additional understanding that Control (0.08 mg Se/day from diet) and Mix (diet plus 1.5 mg supplemental OSe) treatments failed to stimulate BOLA-DQA expression suggests that a threshold amount of supplemental OSe is required that exceeds 1.58 mg of organic Se/day in organic forms.

In contrast to BOLA-DQA2, expression of CEP350 (centromere protein 350kDa) was decreased 1.5- and 1.39-fold by ISe and Mix, respectively. CPE350 (Zimin et al., 2009) is localized to the centrosome and thought to be involved with the

regulation of nuclear hormone receptor function. However, the physiological relevance of decreased CEP 350 expression is not obvious.

Neuropeptide Y is one of the most abundant neuropeptides in the mammalian nervous system and exhibits effects on psychomotor activity, food intake, regulation of central endocrine secretion, and potent vasoactive effects. NPY is found in the liver and can regulate blood flow in, and secretion by, the liver (Ding et al., 1991; El-Salhy, 2000). The receptor for NPY (NPY1R) is expressed by the liver and is associated with glycoprotein processes. Hepatic production of the glycoprotein hormone, which regulates production of bone marrow platelets (<http://review-center.net/metabolism/liver-metabolism-pathways-and-its-disorders/>) and several other glycoproteins, is expressed in fibrotic livers (Li and Friedman, 1999; Tsukada et al., 2006). Aspartate aminotransferase and alanine aminotransferase are hepatic biomarkers associated with NPY1R copy number variation (Kim et al., 2010). That NPY1R was downregulated (1.32-fold) by ISe, but unaffected by Mix or OSe treatments, suggests a decreased regulation of glycoproteins and glycoprotein hormone in liver tissue of ISe heifers.

The SNAPIN is a SNAP-associated protein, and a component of the SNARE complex of proteins that is required for synaptic vesicle docking and fusion (Ilardi et al., 1999). SNAPIN is involved with synaptic vesicle maturation (Pan et al., 2009) and induces synaptic transmission after binding of synaptic vesicles (Ilardi et al., 1999). SNAPIN expression was down-regulated by ISe (1.14-fold) and Mix (1.19-fold), but not by OSe. Thus, ostensibly, heifers consuming ISe or Mix forms of Se would have impaired synaptic transmission capacity relative to Control and OSe heifers, animals consuming only organic forms of Se.

Relationship between DEG and MiRNA expression

The expression of 621 miRNA was detected by miRNA analysis (data not shown). Of these, the expression of three (MiR222, MiR2393, MiR2300b) were affected by Se supplementation treatment. Whereas the expression of MiR222 has been reported in other species, but the expression of MiR2393 and Mir2300b is unique to bovines.

MiR222 (Cluster 6) was up-regulated (1.23-fold) by ISe treatment, but not by the other treatments. Because upregulation of MiR are associated with decreased gene expression, this result suggests that ISe treatment has a negative effect on genes controlled by MiR222. Of the 85 annotated DEG, only seven (GCG, glucagon from Cluster 3; NPY1R, TMEM168, THG1L, THOC5, ADNP2, from Cluster 8) shared this pattern of ISe-induced decreased expression (Table 1). However, none of these are putative targets of MiR222. Instead, only DDHD1 is a putative target (Table 2). However, DDHD1 expression was up-regulated 1.30 fold by Mix treatment (Table 1). Thus, (a) DDHD1 was not a target of MiR222 and (b) the consequence of MiR22 upregulation by Mix Se treatment is not obvious.

In contrast to MiR222, MiR2393 expression was decreased by Se treatment, thus its inhibitory effect of target mRNA would be to increase their expression. Specifically, MIR 2393 (Cluster 4) expression tended to be downregulated (1.20-fold) by ISe and was downregulated by Mix (1.41-fold) and OSe (1.65-fold) (Table 1). However, of the 17 putative targets of MiR2393 (Table 2), one of these, QPCT, was in the same cluster (Cluster 4, any form of Se supplement down regulated DEG as MiR2393 (Table 1). Therefore, QPCT does not appear to be a target of MiR2393.

As for MiR222, DDHD1 also is a target of MiR2393. Unlike MiR222, MiR2393 was downregulated by Mix treatment concomitant with an upregulation of DDHD1. Thus, the data are consistent with DDHD1 expression being regulated by

Mix treatment downregulation of MiR2393, as opposed to regulation by MiR222. DDHD1 can generate the arachidonic acid-containing lysophosphatidylinositol that increases both the phosphorylation of the extracellular signal-regulated kinase (ERK) and intracellular Ca^{2+} levels (Yamashita et al., 2010). TRP5C also is a putative target of MiR2393 whose expression was upregulated by ISe (1.37-fold), Mix (1.58-fold), and OSe (1.67-fold). As noted before, TRP5C (Cluster 1) expression increases the capacity for Ca^{2+} influx into cells (Mori et al., 2011). Together, the concomitant downregulation of MiR2393 and upregulation of DDHD1 and TRP5C by at least Mix and OSe supplementation suggests one mechanism by which Se supplementation of Se-adequate diets results in increased redox potential (Yamamoto et al., 2010). This supposition is strengthened by the observation that SEPW1 (Cluster 1), which functions as an antioxidant enzyme (Jeong et al., 2002), also is upregulated by all three Se supplements (Table 1).

As for MiR2300b, the effect of Se supplementation on liver expression of MiR2300b was to decrease it (Table 1). Thus, the expression of MiR2300b targets should be increased. Unlike MiR2393, however, MiR2300b was down-regulated by ISe (1.25-fold) and Mix (1.37-fold) treatment, not OSe and Mix. Of the putative seven DEG MiR2300b targets (Table 2), four (FRAS1, RUFY3, SNAPIN, THG1L) were downregulated by Se treatment and thus not likely regulated by MiR2300b expression. Of the remaining three putative targets, LRRTM2 is upregulated by ISe and OSe, SNHG3-RCC1 is upregulated by Mix, and SEPW1 is upregulated by all three Se supplement forms. Thus, the expression patterns of none of DEG that are putative targets of MiR2300b match those of MiR2300b, as would be expected in their expression was regulated by MiR2300b.

Predominant Se Treatment-specific Effects on Gene Expression

Although hierarchical clustering of the 139 DEG resulted in the identification of eight different gene expression clusters, consideration of the Se treatment effects on only the 85 annotated DEG (Table 1) reveal four prominent, Se form-induced expression patterns. First, from Clusters 1 (3 genes) and 5 (15 genes), any Se form supplement (ISe, Mix, OSe) increased gene expression relative to Control. Second, in Cluster 3 (15 genes), it is apparent that the Mix treatment upregulates gene expression, whereas OSe or ISe downregulates gene expression. Third, from Cluster 6 (11 genes), whereas OSe does not affect expression, ISe treatment upregulates gene expression and Mix treatment downregulates expression. Fourth, from Clusters 7 (10 genes) and 8 (20 genes) either Mix or ISe treatments downregulate gene expression whereas OSe upregulates expression.

In addition, two other Se form-induced patterns are represented in the gene clusters, but the relative few genes involved with each cluster reduce confidence in their validity. Specifically, from Cluster 2 (3 genes) it would appear that whereas OSe upregulates gene expression, ISe or Mix decreased expression. In contrast, from Cluster 4 (5 genes), whereas one gene is downregulated by all three Se treatments, three others are downregulated by OSe or Mix, but another is upregulated by only ISe treatment.

Comparison of Current to Previous Studies on the Effect of Dietary Se form on Hepatic Gene Expression in Developing Beef Heifers.

Previously, we found that dietary supplementation of none or 3 mg Se/day as ISe or OSe to maturing beef heifers (Liao et al., 2010) altered the expression of about 80 mRNA. Of these, only one DEG (LEPRE1, leucine proline-enriched proteoglycan (leprecan) 1) was found altered by Se supplementation (ISe, OSe) in both experiments. However, in the Liao et al. (2010) trial, LEPRE1 was upregulated by ISe (1.53-fold)

and OSe (1.47-fold) treatments (Mix treatment was not tested), whereas in the present trial, LEPRE1 was not affected by ISe or OSe treatment but was downregulated (1.15-fold) by Mix treatment (which was not used in the Liao et al. (2010) trial).

At first glance, it seems surprising that more similarities were not found between the two studies given that the same experimental model was used. However, upon further consideration, the lack of similarity between gene expression induced by ISe and OSe treatments in the pilot (Liao et al., 2010) versus current trial may be reasonable given the following differences: (1) although similar in magnitude, Se concentrations in liver after 105/106 days of supplementation (Liao et al., 2010) may not have been stable, as they were after 168 days (current trial), (2) different experimental designs were used (non-supplemented Control, ISe, and OSe and $n = 6/\text{treatment}$ for Liao et al., 2010 versus non-supplemented Control, ISe, OSe, and Mix, $n = 9/\text{treatment}$ for the present study), (3) differences in the microarray assays to detect gene expression due to the use of different GeneChips, and (4) a combination of the above. With regard to the third possibility, Liao et al. (2010) used the commercially-available Affymetrix GeneChip Bovine Genome Array, with its 24,016 gene transcripts, and annotated using NetAffix annotation database for 3' IVT Expression on Bovine GeneChip Array 3'-weighted coverage of transcripts (<http://www.affymetrix.com/analysis/index:affx>, Affymetrix, Inc.). In contrast, the present trial used the custom WT Btau 4.0 Array (version 1), which contained 26,303 gene transcripts, a complete 5' to 3' coverage of gene transcripts, and annotation based on an updated draft (4.0) of the Bovine Genome. Of note, the Btau 4.0 GeneChip used in the current study contained 621 microRNA, whereas the Affymetrix GeneChip Bovine Genome Array contained none.

SUMMARY

The expression pattern of these affected genes appeared to be more similar for Control and OSe than Control and ISe, and Mix is between OSe and ISe. This result would suggest that the different gene expression profile of OSe, Mix, ISe heifers, was not caused by an inter-form gradient (0, 50, 100% ISe or OSe) effect, but by their biochemical form and form-specific metabolic pathways.

The affected genes were grouped into eight distinct clusters. Functional analysis of these affected genes by cluster showed that they are associated with different physiological functions, including (1) nutrient metabolism, (2) molecular and mineral transport, (3) cell signaling and interaction, (4) cellular growth, proliferation, (5) immune response and hormone regulation, and (6) tissue/organ development and function. This result would gain a more complete perspective of how different dietary forms of Se caused the specific hepatic transcripts expression and biofunctions profile changed. Regarding the effect of the supplemental Se forms, eight distinct groups of genes were identified: those (1) commonly affected by ISe and OSe supplementation, (2) commonly affected by ISe and Mix supplementation, (3) commonly affected by OSe and Mix supplementation, (4) commonly affected by ISe, OSe, and Mix supplementation, (5) solely affected by ISe supplementation, (6) solely affected by OSe supplementation, (7) solely affected by Mix supplementation, (8) affected by ISe, Mix, or OSe supplementation but not differ from Control.

In addition, the result of the miRNA analysis indicates the possible relationship of the changed hepatic gene expression and biofunctions with the differentially up- or down- regulation of microRNAs caused by different Se supplementation. The true nature of these relationships would still require more

research to fully understand exactly how these microRNAs affect their targets expression.

CONCLUSION

The objectives of the current work were to (1) compare hepatic gene transcriptome profiles when hepatic liver assimilation was stable (d 168) using microarray methods, and (2) conduct bioinformatics analysis of these profiles to predict altered hepatic physiological capacities induced by specific forms of Se supplement.

Hepatic gene transcriptome profiles were successfully compared, resulting in the understanding that the chemical form (inorganic, organic, mix) of supplemental Se differentially affected hepatic gene expression of growing heifers after 168d supplementation. The bioinformatics analysis results indicated that Se treatments induced differentially expressed hepatic genes could be grouped into several clusters upon their expression pattern, and the genes in each cluster are indicative of differentially changed biofunctions. In addition, the putative target mRNAs of Se treatment induced differentially expressed miRNA could influence different biofunctions, but more work needs to be done to validate these findings.

Table 1. Hepatic gene transcripts affected by dietary selenium (Se) supplementation treatment for 168 days to growing beef heifers transcripts

			Dietary Se treatments ²					
Transcript ID	Gene Symbol ³	Gene Description	Control	ISe	Mix	OSe	SEM ⁴	P-value ⁵
Cluster 1								
12907339	TRPC5	transient receptor potential cation channel, subfamily C, member 5	1.00000 ^a	1.36791 ^b	1.58006 ^b	1.66591 ^b	0.12907	0.002725
12697969	LOC100139773	hypothetical LOC100139773	1.00000 ^a	1.15171 ^{a,b}	1.36042 ^b	1.27082 ^b	0.08467	0.006423
12697805	LOC786881	similar to hCG1812167	1.00000 ^a	-1.03848 ^a	1.05093 ^a	1.24424 ^b	0.07294	0.006400
12675620	---Intron	---	1.00000 ^{a,b}	-1.28400 ^b	1.39857 ^a	1.44240 ^a	0.18484	0.006020
12914697	LOC100336032	PREDICTED: Bos taurus hypothetical protein LOC100336032	1.00000 ^a	1.15312 ^{a,b}	1.13500 ^a	1.33547 ^b	0.07299	0.005211
12752300	SEPW1	selenoprotein W, 1	1.00000 ^a	1.22275 ^b	1.21631 ^b	1.26013 ^b	0.05227	0.000289
12710910	NEK5	NIMA (never in mitosis gene a)-related kinase 5	1.00000 ^a	1.00664 ^a	1.08321 ^{a,b}	1.15446 ^b	0.04425	0.007897
12677772	---Intron	---	1.00000 ^a	1.11436 ^a	1.22302 ^a	1.60125 ^b	0.12704	0.006116
Cluster 2								
12914839	LOC100336100	PREDICTED: Bos taurus hypothetical protein LOC100336100	1.00000 ^a	1.15229 ^b	1.18140 ^b	1.03912 ^a	0.04393	0.001174
12881728	PCDHB14	protocadherin beta 14	1.00000 ^a	1.28212 ^b	1.34957 ^b	1.06088 ^a	0.09183	0.006410
12864124	CYP2D14	cytochrome P450, family 2, subfamily D, polypeptide 6	1.00000 ^{a,b}	1.15627 ^a	1.09450 ^a	-1.20399 ^b	0.08664	0.007010
12871032	RUFY3	RUN and FYVE domain containing 3	1.00000 ^a	1.02846 ^a	1.06498 ^a	-1.16205 ^b	0.05771	0.005646
12764375	LOC532807	similar to RUN and FYVE domain containing 3	1.00000 ^a	1.09947 ^{a,b}	1.15836 ^b	-1.09996 ^a	0.06654	0.008889
Cluster 3								
12847828	MGC134040	hypothetical protein MGC134040	1.00000 ^a	-1.03393 ^a	1.17224 ^b	-1.06679 ^a	0.06037	0.005824
12869909	CXCL2	chemokine (C-X-C motif) ligand 2	1.00000 ^a	-1.03514 ^a	1.38450 ^b	-1.13931 ^a	0.11938	0.003537
12693465	CCNB2	cyclin B2	1.00000 ^a	-1.06124 ^a	1.34891 ^b	-1.16518 ^a	0.11918	0.005328
12774328	GCG	glucagon	1.00000 ^a	-1.09897 ^b	1.04313 ^a	-1.06086 ^{a,b}	0.03330	0.001132
12699461	PFN4	profilin family, member 4	1.00000 ^a	1.20285 ^{a,b}	1.33732 ^b	-1.00647 ^a	0.09570	0.007251
12786583	IRX4	iroquois homeobox 4	1.00000 ^a	1.03334 ^a	1.17588 ^b	-1.02677 ^a	0.04733	0.002060
12826590	LOC531024	similar to olfactory receptor 1200	1.00000 ^a	1.06087 ^a	1.21436 ^b	1.07856 ^a	0.03869	0.000243
12686044	LOC537248	similar to ACPL2 protein	1.00000 ^a	1.19225 ^b	1.35993 ^b	1.05331 ^{a,b}	0.07236	0.000661

Table 1. (Continued)

12687785	RNASE12	hypothetical protein LOC100297737	1.00000 ^a	-1.01829 ^a	1.42337 ^b	-1.05685 ^a	0.08304	0.000151
12716554	RIN2	Ras and Rab interactor 2	1.00000 ^a	-1.00644 ^a	1.09313 ^a	-1.15966 ^b	0.05574	0.001630
12782928	SNHG3-RCC1	SNHG3-RCC1 readthrough transcript	1.00000 ^a	1.07997 ^{a,b}	1.15455 ^b	1.02993 ^{a,b}	0.04166	0.009548
12827960	PLAC9	placenta-specific 9	1.00000 ^a	1.13081 ^{a,b}	1.32674 ^b	1.10323 ^{a,b}	0.05955	0.000667
12900046	LOC510904	hypothetical LOC510904	1.00000 ^a	1.32197 ^b	1.33335 ^b	1.23397 ^b	0.08776	0.006865
12905415	LOC539973	similar to Zinc finger, DHHC-type containing 3	1.00000 ^a	1.08064 ^a	1.32775 ^b	1.08349 ^a	0.07614	0.007144
12729265	MTMR2	myotubularin related protein 2	1.00000 ^{a,b}	-1.02685 ^a	1.09178 ^c	1.03129 ^b	0.02711	0.001488
12706499	PSME4	proteasome (prosome, macropain) activator subunit 4	1.00000 ^a	1.02009 ^a	1.19114 ^b	1.00366 ^a	0.05130	0.003979
12676376	---Intron	---	1.00000 ^a	1.01106 ^a	1.30861 ^b	1.32247 ^b	0.10309	0.009497
12803871	LOC782475	similar to olfactory receptor 1368	1.00000 ^a	-1.13553 ^a	1.36319 ^b	1.09288 ^{a,b}	0.11226	0.005933
12910859	eIf4a1	eukaryotic translation initiation factor 4A1	1.00000 ^a	1.00747 ^a	1.21265 ^b	1.10533 ^{a,b}	0.05802	0.007803
12814476	ANKRD61	ankyrin repeat domain 61	1.00000 ^a	1.02440 ^a	1.21770 ^b	1.08275 ^a	0.05766	0.007762
12677234	---Intron	---	1.00000 ^a	1.26833 ^a	1.71743 ^b	1.12841 ^a	0.14605	0.005896
12693689	DDHD1	DDHD domain containing 1	1.00000 ^a	1.04630 ^a	1.29974 ^b	1.02109 ^a	0.06566	0.001069
12675450	---Intron	---	1.00000 ^{a,c}	-1.43052 ^b	1.21088 ^c	-1.22692 ^{a,b}	0.15143	0.008101
Cluster 4								
12677448	---Intron	---	1.00000 ^a	1.13538 ^a	-1.37222 ^b	-1.39136 ^b	0.13136	0.003895
12676664	---Intron	---	1.00000 ^a	-1.28715 ^{a,b}	-1.38955 ^{b,c}	-1.80657 ^c	0.15769	0.006690
12765783	GSG2	germ cell associated 2 (haspin)	1.00000 ^a	-1.02219 ^a	-1.03877 ^a	-1.23345 ^b	0.06242	0.008242
12888519	LOC788287	similar to Olfactory receptor 90	1.00000 ^a	1.03204 ^a	-1.04864 ^a	-1.18675 ^b	0.05684	0.005632
12698464	QPCT	glutaminyl-peptide cyclotransferase	1.00000 ^a	-1.16423 ^b	-1.26451 ^b	-1.29939 ^b	0.06257	0.001040
12818810	MIR2393	microRNA mir-2393	1.00000 ^a	-1.20463 ^{a,b}	-1.41205 ^{b,c}	-1.65305 ^c	0.09321	0.000119
12911831	ZNF238	Zinc finger 238 (RP58)	1.00000 ^a	1.02814 ^a	-1.10135 ^{a,b}	-1.17690 ^b	0.05856	0.008190
12686393	MFI2	antigen p97 (melanoma associated) identified by monoclonal antigen	1.00000 ^{a,b}	1.07395 ^b	-1.06797 ^a	-1.10413 ^a	0.04999	0.009302
12676594	---Intron	---	1.00000 ^a	1.05904 ^a	1.13670 ^a	-1.26234 ^b	0.09904	0.005182
Cluster 5								
12796744	TTC21A	tetratricopeptide repeat domain 21A	1.00000 ^a	1.21548 ^b	1.01361 ^a	1.02297 ^a	0.06029	0.007711
12912929	CAPRIN2	caprin family member 2	1.00000 ^a	1.13153 ^b	-1.02243 ^a	-1.05430 ^a	0.04810	0.006433

Table 1. (Continued)

12914495	LOC516089(discon)	PREDICTED: Bos taurus olfactory receptor 78-like	1.00000 ^a	1.25318 ^b	1.15570 ^b	1.12760 ^b	0.05707	0.004526
12879404	FGF22	fibroblast growth factor 22	1.00000 ^a	1.29373 ^b	1.08106 ^a	1.08686 ^a	0.07032	0.006865
12873243	CNGA1	cyclic nucleotide gated channel alpha 1	1.00000 ^a	1.19197 ^b	1.13980 ^b	1.12575 ^b	0.04591	0.004660
12857050	LALBA	lactalbumin, alpha	1.00000 ^a	1.23641 ^b	1.20520 ^b	1.02791 ^a	0.06083	0.001969
12705807	ABO	ABO blood group (transferase A, alpha 1-3-N-acetylgalactosaminy	1.00000 ^a	1.22678 ^b	1.10102 ^{ab}	1.02565 ^a	0.05988	0.007013
12726472	LOC100300575	similar to olfactory receptor 154	1.00000 ^a	1.25720 ^c	1.20417 ^{b,c}	1.08372 ^{ab}	0.05796	0.002456
12807139	LOC786846	similar to olfactory receptor 108	1.00000 ^a	1.18358 ^b	1.14196 ^{b,c}	1.02988 ^{a,c}	0.05462	0.009475
12882374	PCDHA2	protocadherin alpha 2	1.00000 ^a	1.16689 ^b	1.02240 ^a	-1.00409 ^a	0.04904	0.008942
12911511	ADAM7	Disintegrin and metalloproteinase domain-containing protein 7-like	1.00000 ^a	1.29932 ^b	1.16221 ^b	1.16758 ^b	0.05890	0.001454
12834487	NTM	neurotrimin	1.00000 ^a	1.25025 ^b	1.18765 ^b	1.17953 ^b	0.06262	0.007055
12786958	ITGA2	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)	1.00000 ^a	1.27304 ^b	1.22285 ^b	1.33601 ^b	0.07739	0.004259
12720027	CDH4	cadherin 4, type 1, R-cadherin (retinal)	1.00000 ^a	1.19777 ^b	1.22550 ^b	1.10903 ^{ab}	0.06082	0.009903
12691885	LOC614591	similar to Olfr736 protein	1.00000 ^a	1.31777 ^b	1.07387 ^a	-1.01174 ^a	0.07211	0.001495
12747236	LOC531152	similar to solute carrier family 5 (sodium/glucose cotran	1.00000 ^a	1.17262 ^b	1.08239 ^{ab}	1.02624 ^a	0.04312	0.004690
12687141	LOC781919	hypothetical LOC781919	1.00000 ^a	1.29038 ^b	1.09990 ^a	-1.02790 ^a	0.07560	0.003120
12831279	LRFN4	leucine rich repeat and fibronectin type III domain containin	1.00000 ^a	1.25464 ^b	1.20157 ^b	1.07978 ^b	0.06154	0.003386
12693387	LOC100337247	hCG1812119-like	1.00000 ^a	1.33301 ^b	1.22073 ^b	-1.02350 ^a	0.09509	0.005659
12885640	SHD	Src homology 2 domain containing transforming protein D	1.00000 ^a	1.17028 ^b	1.16044 ^b	1.07479 ^{ab}	0.04725	0.006491
12843025	SLC6A17	solute carrier family 6, member 17	1.00000 ^a	1.26507 ^b	1.23217 ^b	1.11242 ^{ab}	0.06281	0.002701
12906697	LOC100336642	hypothetical protein LOC100336642	1.00000 ^a	1.19347 ^b	1.23236 ^b	1.07057 ^{ab}	0.05958	0.004127
12707153	LYG2	lysozyme G-like 2	1.00000 ^a	1.10830 ^{ab}	1.22910 ^b	1.05533 ^{ab}	0.05476	0.004287
12713813	MMP9	matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92k	1.00000 ^a	1.24610 ^b	1.20563 ^b	1.08945 ^{ab}	0.05874	0.002801
12842299	GJB4	gap junction protein, beta 4, 30.3kDa	1.00000 ^a	1.13385 ^b	1.26389 ^b	1.10493 ^{ab}	0.05783	0.003008
12910332	LOC618023	similar to mCG1035526	1.00000 ^a	1.13489 ^b	1.16805 ^b	1.00666 ^a	0.05158	0.006880
12829684	CEND1	cell cycle exit and neuronal differentiation 1	1.00000 ^a	1.20278 ^b	1.10631 ^{ab}	-1.04940 ^a	0.05132	0.000338
12911453	LOC617745	PREDICTED: Bos taurus histone cluster 1, H2bd-like	1.00000 ^a	1.33297 ^b	1.26593 ^b	1.02369 ^a	0.08867	0.004406

Table 1. (Continued)

12677810	---Intron	---	1.00000 ^a	1.80027 ^b	1.44397 ^{a,b}	1.01137 ^a	0.18686	0.007738
Cluster 6								
12677714	---Intron	---	1.00000 ^a	1.25973 ^b	1.04358 ^a	1.23621 ^b	0.07479	0.006589
12802414	LOC523769	similar to Olfactory receptor 11A1 (Olfactory receptor OR	1.00000 ^a	1.33145 ^b	1.17429 ^b	1.17281 ^b	0.07550	0.008159
12741683	LOC100295022	similar to pre-B lymphocyte gene 2	1.00000 ^{a,c}	1.12993 ^{a,b}	-1.06419 ^c	1.17265 ^b	0.06415	0.005043
12740074	LOC530749	hypothetical LOC530749	1.00000 ^a	1.18507 ^b	-1.01920 ^a	1.08987 ^{a,b}	0.05729	0.008473
12804717	GCM1	glial cells missing homolog 1 (Drosophila)	1.00000 ^a	1.20950 ^b	1.04205 ^a	1.05058 ^a	0.05491	0.009211
12686542	C1H21ORF7	chromosome 21 open reading frame 7 ortholog	1.00000 ^a	1.04049 ^a	-1.16979 ^b	-1.03255 ^a	0.05544	0.006607
12906283	MIR222	microRNA mir-222	1.00000 ^a	1.22316 ^b	-1.00025 ^a	-1.09591 ^a	0.06853	0.001913
12824553	ZNF703	zinc finger protein 703	1.00000 ^a	1.14629 ^b	-1.04146 ^a	1.03012 ^a	0.04940	0.007012
12730430	LOC788649	similar to odorant receptor HOR3beta3	1.00000 ^a	1.22925 ^b	-1.00493 ^a	1.07249 ^a	0.06580	0.008928
12911627	LOC790172	PREDICTED: Bos taurus membrane glycoprotein gp140-like	1.00000 ^a	1.25359 ^b	1.10068 ^{a,c}	1.15272 ^{b,c}	0.05657	0.006471
12713247	LOC100196900	hypothetical LOC100196900	1.00000 ^a	1.09330 ^a	-1.00273 ^a	-1.18405 ^b	0.07243	0.008923
12866241	TULP3	tubby like protein 3	1.00000 ^a	1.06038 ^a	-1.11448 ^b	-1.00561 ^a	0.04430	0.007012
12876076	LOC782977	similar to pol protein	1.00000 ^a	1.16389 ^b	-1.10204 ^a	1.06022 ^{a,b}	0.06658	0.008782
12913095	PAKD1	---	1.00000 ^a	1.74958 ^b	1.04876 ^a	1.04799 ^a	0.15558	0.003061
12902043	LOC100298746	similar to protein phosphatase 2, regulatory subunit	1.00000 ^a	1.17319 ^b	1.00527 ^a	1.02182 ^a	0.04358	0.001923
12882371	OR7E24	similar to Olfactory receptor 18 (Olfactory receptor 145-	1.00000 ^a	1.42674 ^b	1.01118 ^a	1.06376 ^a	0.08860	0.001017
12854700	FBXL13	F-box and leucine-rich repeat protein 13	1.00000 ^a	1.16934 ^b	-1.03845 ^a	1.03846 ^a	0.05034	0.003826
12717977	GHRH	growth hormone releasing hormone	1.00000 ^a	1.22927 ^b	1.06399 ^a	-1.00427 ^a	0.06520	0.009874
12712598	PDX1	pancreatic and duodenal homeobox 1	1.00000 ^a	1.16694 ^b	-1.02570 ^a	1.02967 ^a	0.05157	0.006236
12682969	LOC100296558	hypothetical protein LOC100296558	1.00000 ^a	1.41912 ^b	1.15929 ^a	1.08157 ^a	0.07247	0.000266
12674910	---Intron	---	1.00000 ^a	1.35052 ^b	-1.21602 ^a	1.12980 ^a	0.11494	0.001786
12729575	HBE1	hemoglobin, epsilon 1	1.00000 ^a	1.07921 ^{a,b}	-1.14303 ^b	-1.10060 ^{a,b}	0.06238	0.008809
Cluster 7								
12856863	LDHB	lactate dehydrogenase B	1.00000 ^a	-1.02584 ^a	-1.13592 ^a	1.21088 ^b	0.08583	0.007247
12707574	VAMP5	vesicle-associated membrane protein 5 (myobrevin)	1.00000 ^a	1.09221 ^{a,b}	1.01750 ^{a,b}	1.18502 ^b	0.04999	0.008498
12906833	LOC100337148	nuclear RNA export factor 3-like	1.00000 ^a	1.20167 ^b	1.03651 ^{a,b}	1.34291 ^b	0.08945	0.008903

Table 1. (Continued)

12675356	---Intron	---	1.00000 ^a	1.10150 ^a	-1.43145 ^b	1.14442 ^a	0.10746	0.000423
12844091	TNFAIP8L2	tumor necrosis factor, alpha-induced protein 8-like 2	1.00000 ^a	1.02130 ^a	-1.10577 ^b	1.07832 ^a	0.04659	0.007207
12770586	CDRT4	CMT1A duplicated region transcript 4	1.00000 ^a	-1.04470 ^{a,b}	-1.15988 ^b	1.06567 ^{a,b}	0.06043	0.009912
12676756	---Intron	---	1.00000 ^a	1.13462 ^{a,b}	-1.12408 ^a	1.22693 ^b	0.09456	0.009437
12885754	LRRTM2	leucine rich repeat transmembrane neuronal 2	1.00000 ^a	1.17937 ^b	-1.03318 ^a	1.12832 ^b	0.05972	0.005353
12675362	---Intron	---	1.00000 ^a	-1.15313 ^{a,b}	-1.42605 ^c	-1.24480 ^{b,c}	0.09101	0.004356
12678012	---Intron	---	1.00000 ^a	-1.00494 ^a	-1.26902 ^b	-1.10875 ^a	0.06429	0.002107
12857735	PLCZ1	phospholipase C, zeta 1	1.00000 ^a	-1.06351 ^{a,b}	-1.19444 ^c	-1.13876 ^{b,c}	0.04580	0.002755
12910700	MCART6	similar to mitochondrial carrier triple repeat 6	1.00000 ^a	1.05026 ^a	-1.17399 ^b	1.01611 ^a	0.06033	0.006992
12871908	FRAS1	similar to rCG64566	1.00000 ^a	1.03201 ^a	-1.17755 ^b	-1.00326 ^a	0.05950	0.009642
12895450	SLC35D2	solute carrier family 35, member D2	1.00000 ^a	-1.09449 ^b	-1.13431 ^b	1.00502 ^a	0.04307	0.008158
12732657	OR2AT4	similar to olfactory receptor Olr35	1.00000 ^a	-1.03373 ^{a,b}	-1.11814 ^b	1.06801 ^a	0.04643	0.007303
12759892	LOC785933	similar to Orphan sodium- and chloride-dependent neurotra	1.00000 ^a	1.01002 ^{a,b}	-1.14507 ^a	1.15762 ^b	0.06967	0.004500
12914365	LOC100462699	---	1.00000 ^{a,b}	1.23788 ^b	-1.26864 ^a	1.13590 ^b	0.11736	0.004271
Cluster 8								
12915559	BOLA-DQA2	Bos taurus major histocompatibility complex, class II	1.00000 ^a	-1.30149 ^a	-1.21918 ^a	2.66633 ^b	0.37359	0.008451
12870473	NPY1R	neuropeptide Y receptor Y1	1.00000 ^a	-1.31851 ^b	-1.11101 ^a	1.06292 ^a	0.07942	0.001029
12851703	TMEM168	transmembrane protein 168	1.00000 ^{a,b}	-1.04902 ^b	1.07443 ^a	1.07428 ^a	0.03587	0.005384
12677980	---Intron	---	1.00000 ^a	-1.26612 ^b	-1.11131 ^{a,b}	1.20419 ^a	0.10765	0.004477
12805856	LOC521580	similar to histone cluster 1, H2bd	1.00000 ^a	1.03429 ^a	1.04456 ^a	1.65795 ^b	0.15039	0.004848
12913565	---PLOC1	---	1.00000 ^{a,b}	-1.16906 ^a	-1.07681 ^a	1.22793 ^b	0.10040	0.008681
12899187	LOC100297846	similar to trace amine associated receptor 8	1.00000 ^a	-1.03889 ^a	1.06342 ^a	1.20792 ^b	0.05900	0.002913
12730199	LOC782645	similar to olfactory receptor Olr245	1.00000 ^a	1.02972 ^a	1.12569 ^{a,b}	1.25095 ^b	0.05992	0.003096
12810320	ZNF236	zinc finger protein 236	1.00000 ^{a,b}	-1.06458 ^a	-1.03225 ^a	1.05678 ^b	0.03261	0.007426
12878036	THG1L	tRNA-histidine guanylyltransferase 1-like (S. cerevisiae)	1.00000 ^a	-1.14203 ^b	-1.07103 ^{a,b}	-1.00782 ^a	0.03735	0.003804
12708470	LOC782479	similar to ribosomal protein L26	1.00000 ^a	-1.21664 ^b	-1.06766 ^{a,b}	1.11736 ^a	0.08608	0.009312
12870694	SRP72	signal recognition particle 72kDa	1.00000 ^{a,b}	-1.02321 ^a	-1.08373 ^a	1.08183 ^b	0.04071	0.006627
12857895	PUS7L	pseudouridylate synthase 7 homolog (S. cerevisiae)	1.00000 ^a	-1.10623 ^a	-1.09610 ^a	1.13670 ^b	0.05312	0.000423

Table 1. (Continued)

12743540	GOLGA3	golgi autoantigen, golgin subfamily a, 3	1.00000 ^a	-1.06641 ^{ab}	-1.08873 ^b	1.08322 ^a	0.03868	0.000934
12842784	FCGR2	Fc fragment of IgG, low affinity IIb, receptor (CD32)	1.00000 ^a	-1.13416 ^{ab}	-1.27149 ^b	1.13919 ^a	0.08708	0.001204
12747334	THOC5	THO complex 5	1.00000 ^a	-1.08233 ^b	-1.03511 ^{ab}	1.03841 ^a	0.03025	0.004399
12809240	ADNP2	ADNP homeobox 2	1.00000 ^a	-1.10483 ^b	-1.01514 ^a	1.06486 ^a	0.03198	0.000331
12844243	SNAPIN	SNAP-associated protein	1.00000 ^a	-1.14259 ^b	-1.19432 ^b	-1.07056 ^{ab}	0.05038	0.007386
12828917	ZNF22	zinc finger protein 22 (KOX 15)	1.00000 ^a	-1.14634 ^b	-1.18867 ^b	-1.07987 ^{ab}	0.04388	0.002467
12690686	ACIN1	apoptotic chromatin condensation inducer 1	1.00000 ^a	-1.07579 ^b	-1.11190 ^b	-1.01875 ^{ab}	0.02836	0.002180
12913489	ACLA_046210	DnaJ domain protein	1.00000 ^a	-1.18778 ^{ab}	-1.40734 ^b	1.12130 ^a	0.11243	0.002185
12840769	LEPRE1	leucine proline-enriched proteoglycan (leprecan) 1	1.00000 ^a	-1.08090 ^{ab}	-1.11548 ^b	1.05989 ^a	0.04569	0.003257
12677698	---Intron	---	1.00000 ^a	-1.12940 ^a	-1.06740 ^a	1.15727 ^b	0.06441	0.001365
12912371	GCLC	glutamate-cysteine ligase, catalytic subunit	1.00000 ^a	-1.24385 ^b	-1.19484 ^b	-1.23431 ^b	0.06410	0.005740
12913085	CEP350	centromere-associated protein 350	1.00000 ^a	-1.56052 ^b	-1.38906 ^b	-1.08920 ^a	0.10649	0.000839
12708824	MIR2300B	microRNA mir-2300b	1.00000 ^a	-1.24583 ^b	-1.32703 ^b	-1.03539 ^a	0.07937	0.001808

¹Se supplement treatments that contained no Se (Control) or 3 mg Se/day in the form of sodium selenite (ISe), 1.5 mg Se/day of each sodium selenite and SelPlex[®] mix (Mix), or 3 mg Se/day of SelPlex[®] (OSe) were top-dressed onto enough of a common cottonseed hull/soybean hull/cracked corn-based diet (0.08 mg Se per day) to support 0.5 kg/day growth in maturing Angus-cross heifers for 168 days. The abundance of gene transcripts are reported relative to the mean expression of the Control group and are expressed as the fold-change of non-transformed data. ²Means with different superscripted letters differ (P < 0.05).

³A missing symbol indicates a lack of confirmed bovine DNA or RNA sequence annotation.

⁴The presented SEM values were pooled (averaged) from that of control (n = 9), ISe (n = 9), Mix (n = 9), and OSe (n = 9) treatment groups.

⁵P-values were obtained from one-way ANOVA F-test

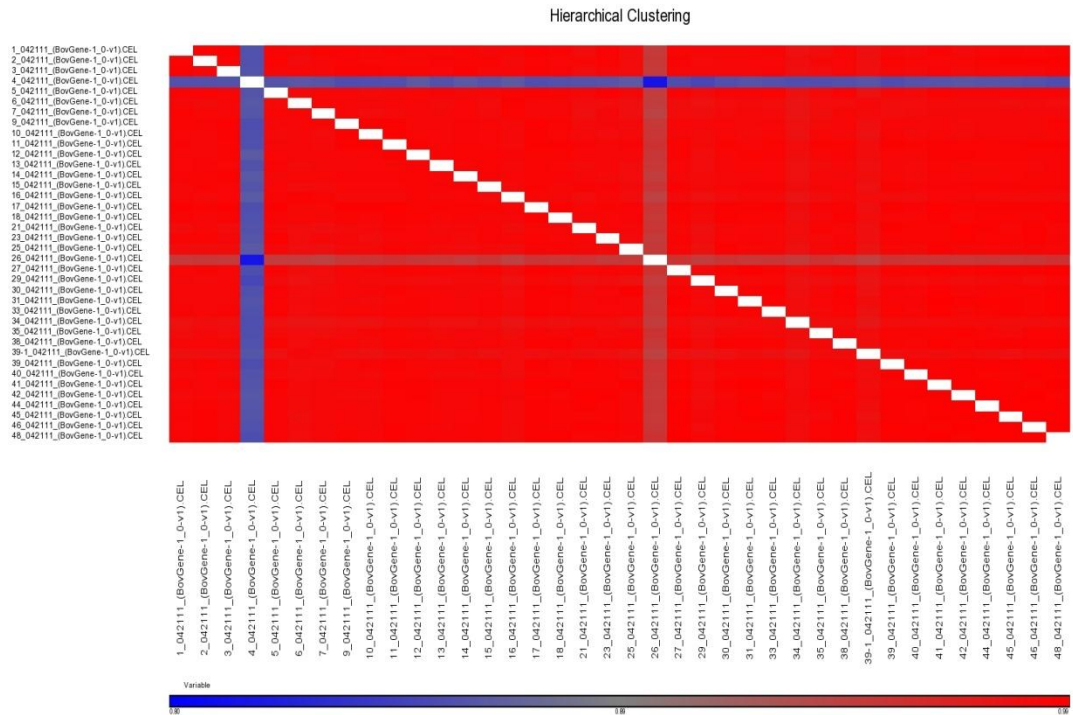
Table 2. Hepatic miRNA (Mir), and their putative mRNA targets, that were differentially expressed in response to dietary Se supplement.¹

MiR	Molecules in Network ²	Top Functions ³
Mir-222	ADCYAP1, <u>DDHD1</u>	Cell Morphology, Cell-To-Cell Signaling and Interaction, Cellular Assembly and Organization
Mir-2393	ABCF3, <u>ACIN1</u> , AKR1C3, CASP2, CBX1, CBX3, CCNA1, CCND1, CDKN2A, CLDN2, CTNNB1, DIABLO, <u>GCM1</u> , <u>GOLGA3</u> , GSK3B, HDAC1, HIC1, HNF1A, HNF4A, HTRA2, <u>LRRTM2</u> , <u>MFI2</u> , NFYC, PPARA, <u>QPCT</u> , RBM39, <u>RIN2</u> , <u>RUFY3</u> , SAP18, SENP1, SRPK2, SRR, TANK, TSNAX, <u>ZNF236</u>	Cell Cycle, Cancer, Neurological Disease
	ADA, ADCYAP1, anandamide, Ca ²⁺ , CCL22, chemokine, CIB1, <u>CXCL2</u> , CXCR2, D-sphingosine, <u>DDHD1</u> , ENTPD1, Eotaxin, FCGR2A, FKBP4, HOMER1, Integrin alpha 4 beta 1, <u>ITGA2</u> , ITPR3, LAMA1, leukotriene B4, Lfa-1, MOG, Plcbeta, PTAFR, Rap1, SELPLG, SFTPD, SLC9A3R1, TRP, TRPC3, TRPC4, <u>TRPC5</u> , VIPR1, Vla-4	Cell Signaling, Molecular Transport, Vitamin and Mineral Metabolism
	CRK, <u>PCDHA2</u>	Embryonic Development, Organ Development, Organismal Development
	E2F1, <u>ZNF22</u>	Cancer, Cardiac Dysfunction, Cell Cycle
	DLG4, <u>FRAS1</u> , GRIP1, GRIP2	Developmental Disorder, Genetic Disorder, Ophthalmic Disease
	<u>ADNP2</u> , CBX1, CBX3, NFYC	DNA Replication, Recombination, and Repair, Gene Expression, Cellular Assembly and Organization
Mir-2300b	BCAR3, CCND1, CDK4, CSNK1A1, CSNK1D, CSNK1E, CTNNB1, DDR1, dihydrotestosterone, DYNC1I1, EBAG9, HADHB, HIST2H2BE, KAT7, KPN A4, <u>LRRTM2</u> , MAPK14, NDC80, NGFR, NPY, PCM1, PKM2, RALY, RANBP1, <u>RCC1</u> , RGS7, <u>RUFY3</u> , <u>SNAPIN</u> , TP53, TRPV1, Tubulin, UNC119, VRK1, XPO1, ZFP106	Cell Cycle, Cellular Assembly and Organization, DNA Replication, Recombination, and Repair
	ELMOD1, <u>THG1L</u>	Tissue Morphology, Cellular Growth and Proliferation, Cell Death
	DLG4, <u>FRAS1</u> , GRIP1, GRIP2	Developmental Disorder, Genetic Disorder, Ophthalmic Disease
	CTNNB1, dihydrotestosterone, SECISBP2, <u>SEPW1</u>	Organismal Injury and Abnormalities, Renal Damage, Renal and Urological Disease

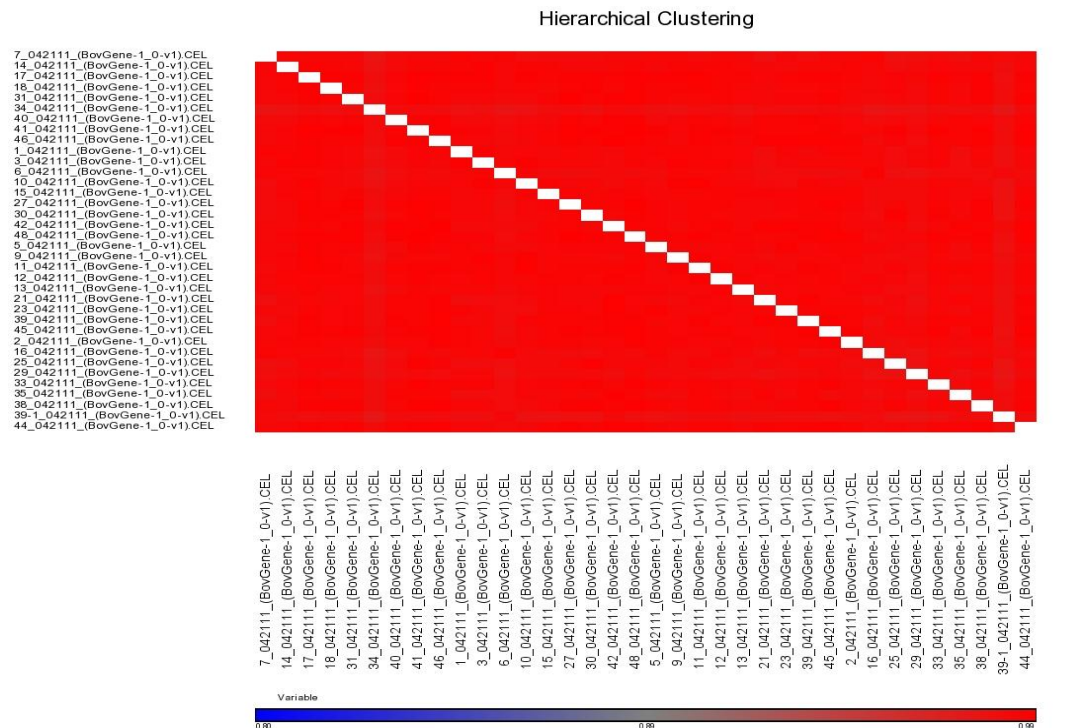
¹Differences in expression and dietary Se treatments are described in Table 1.

²The mRNA for these proteins were differentially expressed by the liver in response to supplemental Se treatment.

³The top biofunctions are identified for the differentially expressed genes in the left.



A



B

Figure 1. The correlation heat maps of 38 *. Cel files (A) before and 36 *.cel files (B) after removing two outliers. The heat maps were generated based on the correlation matrix (data are not shown). As indicated by the legend color box, gray color in the middle represents the correlation coefficient value, 0.90, red color on the rightmost

represents the highest correlation, and the blue color on the leftmost represents the lowest correlation. The intensity of the color reflects the relative intensity of correlation among chips. 8a indicates two chips are not correlated very well compared to other chips. Their individual average correlation coefficients are 0.84 and 0.94. After removing these two chips, 8b indicates the rest chips are correlated very well.

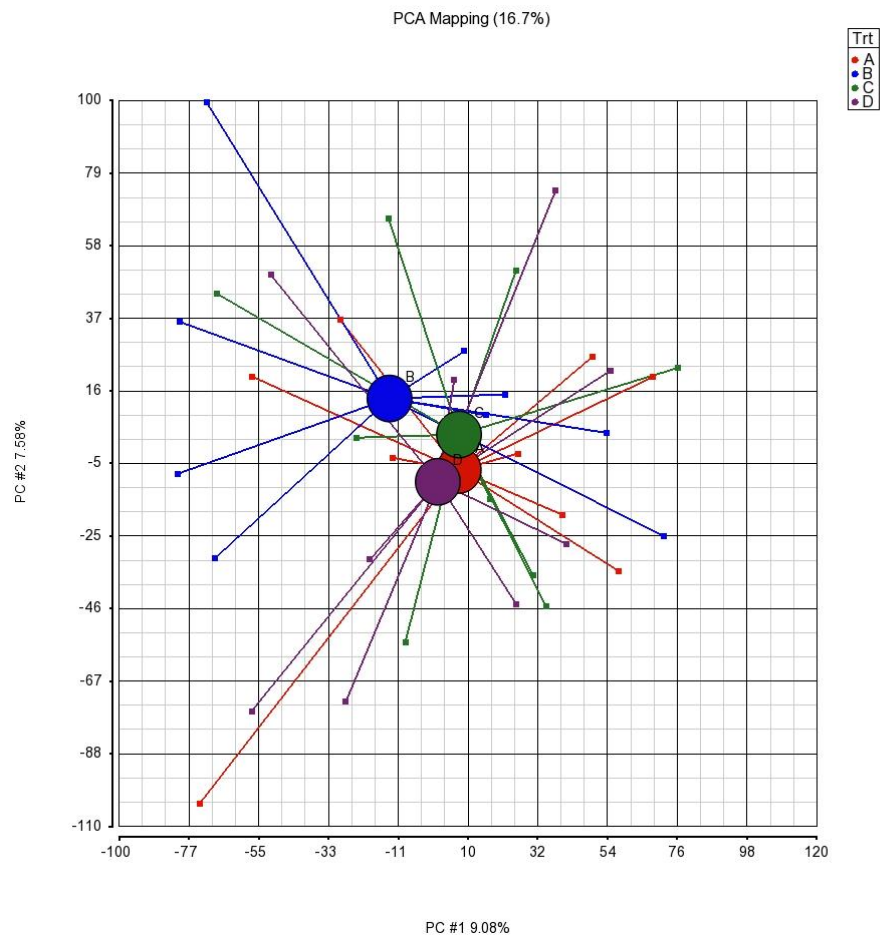


Figure 2. Principle component analysis of microarray transcriptome analysis of day 168 liver samples at day 168 from Control (A/red), ISe (B/blue), Mix (C/green), and OSe (D/purple) maturing Angus-cross heifers. The colored dots represent linear combinations of the relative expression data, including expression values and variances, of the 26,303 gene transcripts in each Bovine GeneChip. The center dot (centroid) for each treatment groups represents the overall treatment expression pattern.

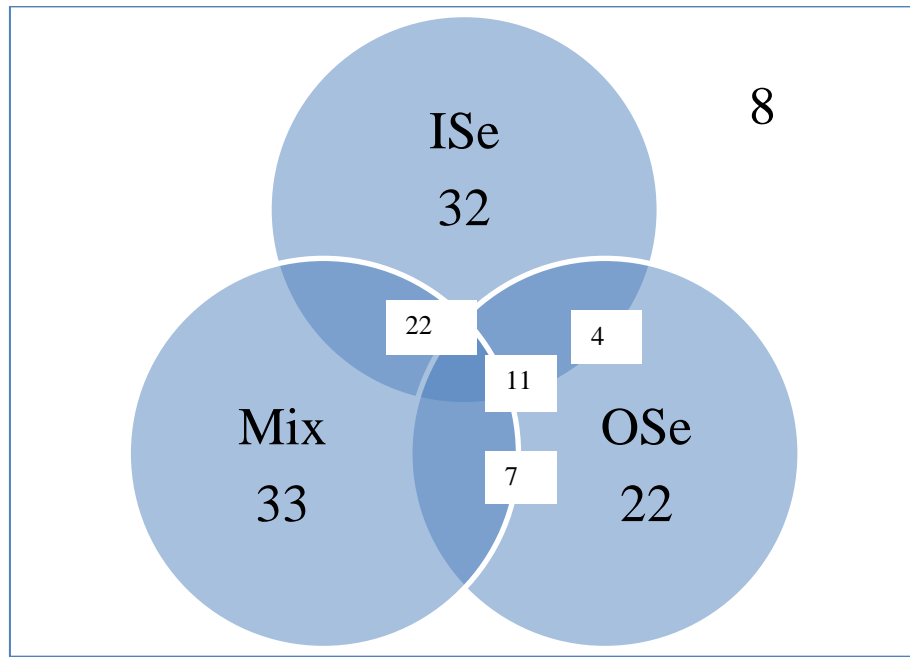


Figure 3. Zen diagram depiction of the relationship of 139 differentially expressed gene transcripts by liver tissue of maturing Angus-cross heifers after 168 days of no supplemental Se (Control) or 3 mg Se/day in the form of sodium selenite (ISe), 1.5 mg Se/day of each sodium selenite and SelPlex[®] mix (Mix), or 3 mg Se/day of SelPlex[®] (OSe). Note that expression of eight transcripts differed among ISe, Mix, or OSe treatments, but not versus Control heifers.

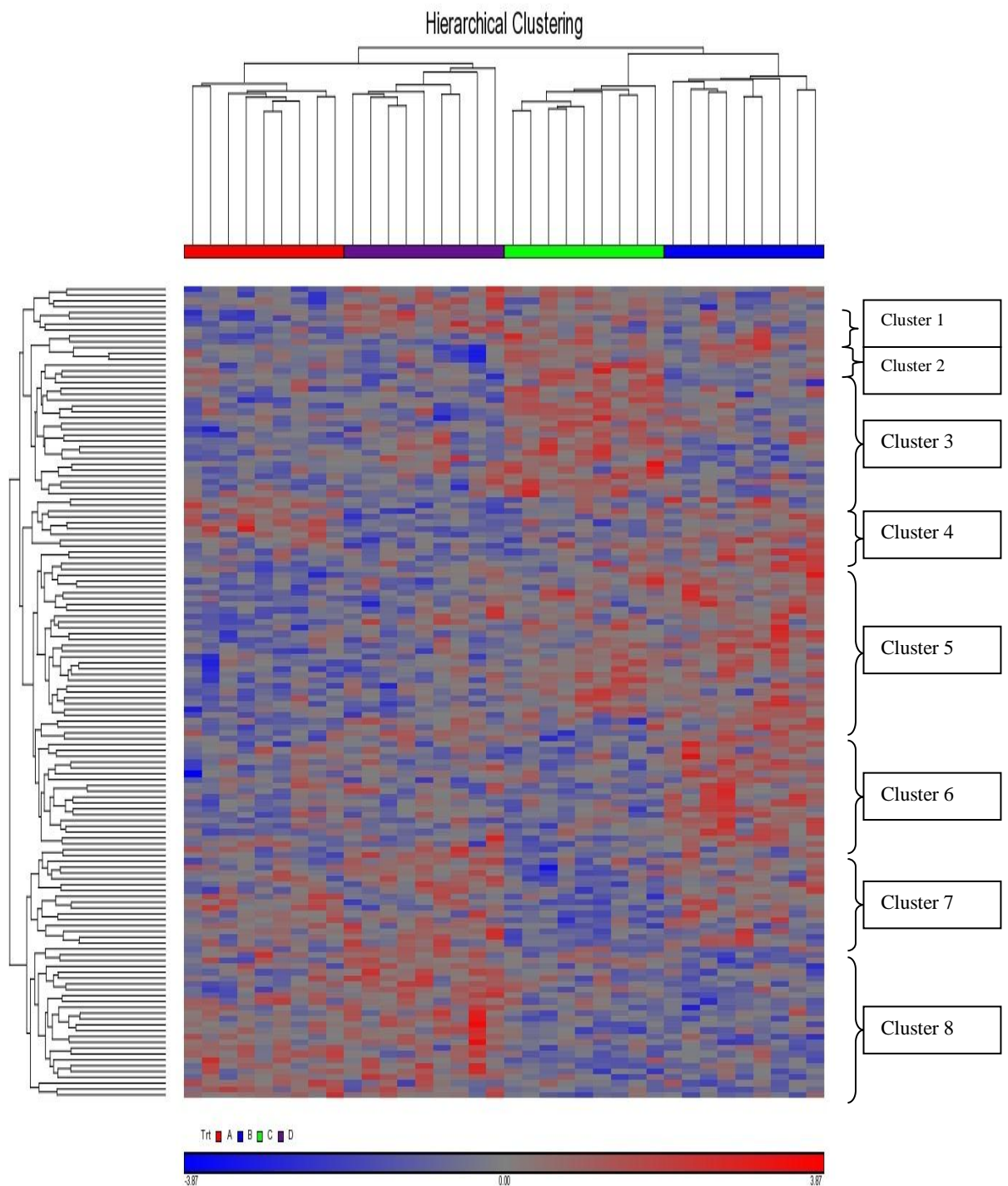


Figure 4. Hierarchical cluster analysis of 139 differentially expressed ($P < 0.01$) gene transcripts by liver tissue of maturing Angus-cross heifers after 168 days of no supplemental Se (Trt A, red) or 3 mg Se/day sodium selenite (Trt B, blue), 1.5 mg Se/day of each sodium selenite and SelPlex[®] mix (Trt C, green), or 3 mg Se/day in the

form of SelPlex[®] (Trt D, purple), as indicated by the top color bar. The expression level for each gene transcript was standardized to mean of 0 and scale to standard deviation of 1, which is the default setting of the Partek Genomics Suite software. As indicated by the bottom color bar, gray color in the middle represents the mean value, 0, red color represents gene expression levels above the mean expression whereas blue color denotes expression below the mean. The intensity of the color reflects the relative intensity level of transcript expression.

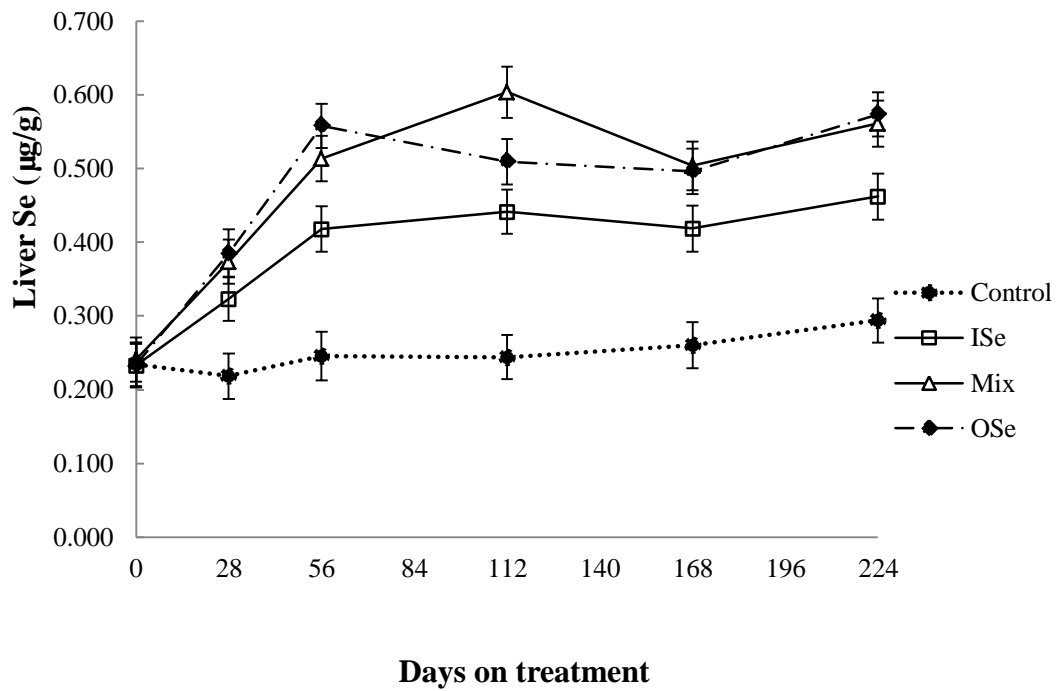


Fig. 5. Liver selenium (Se) concentrations (lsmeans with SEM as error bars) in maturing beef heifers fed diets (224 days) supplemented with either no Se (Control) or 3 mg Se/day in the form of sodium selenite (ISe), sodium selenite/SelPlex[®] mix (Mix) or SelPlex[®] (OSe). Treatment, days on treatment, and treatment x days on treatment interaction effects ($P \leq 0.0001$). Taken from Brennan et al.(2011).

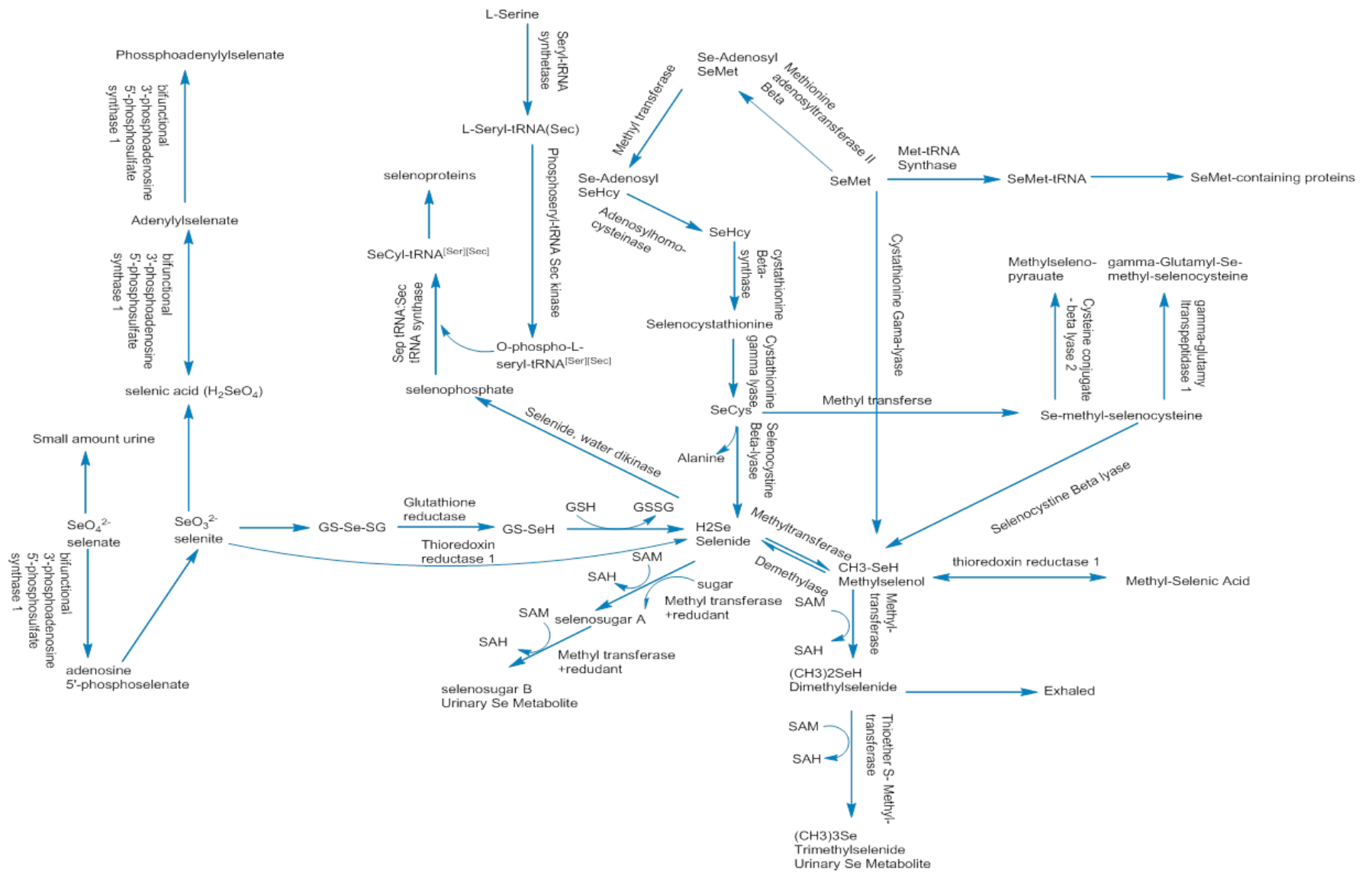


Figure 6. The pathway of different forms of Se metabolism. The whole figure shows the details of the pathway through which Se obtained as dietary SeMet, SeCys, Selenate, and Selenite. After absorption and transport to the liver, they all are transformed to the selenide intermediate that is incorporated into selenoproteins or excreted through methylation (Suzuki, 2005). The routes they come to Selenide are different. Inorganic Se as Selenate and selenite must be reduced, either directly through the action of thioredoxin reductase plus thioredoxin, or react with GSH to form GS-Se-SG, then form GS-SeH by the GSH reductase, then forms selenide. (Lu J et al., 2009). In mammals, the selenate first be converted to selenite than participate the incorporation of selenoproteins. The conversion is catalyzed by the bifunctional enzyme (fusion product of two catalytic activities) involved two steps: sulfate adenylyltransferase catalyzes the formation of adenosine 5'-phosphosulfate (APS) from ATP and inorganic sulfate, then the APS is catalyzed by the adenylylsulfate kinase portion of 3'-phosphoadenosine 5'-phosphosulfate (PAPS) synthase to synthesize the selenite (Bandurski et al., 1956; Hilz and Lipmann, 1955; Venkatachalam et al., 1998). The selenite can also be converted back to selenic acid, the latter will be adenylylated to adenylylselenate then phosphorlated to phosphoadenylylselenate catalyzed by the bifunctional enzyme phosphoadenosine-phosphosulfate synthase (Lehninger, 2005; Salway, 2004). SeCys was through one step to be converted to selenide by the SeCys beta-lyase, with the production of Alanine (Ohta and Suzuki, 2008). Besides, the methyltransferases can transfer one-carbon groups to SeCys to become methylSeCys, the later will be converted to gamma-glutamyl-Se-methyl-SeCys, methyl-seleno-pyruvate, or methylselenol (Kegg, 2011). SeMet uses two paths to go into the Selenide. The primary one is by transselenation to convert into SeCys like the Met transfers to Cys (Suzuki, 2005), the intermediate Se-SAM was synthesized (Gammelgaard et al., 2011), this process is shown in the top.

The other one is by a gamma-lyase catalyzed reaction to produce methylselenol, then by a demethylation process to produce selenide (Suzuki, 2005). SeMet is also incorporated nonspecifically in place of Met into proteins, which is shown on the right top (review paper). Once selenide is prepared, it is either used for synthesis of selenoproteins (on the left top of Figure) or as an intermediate metabolite for Se excretion (on the bottom of Figure 6). Further steps in the assimilation of selenide into selenoproteins involve generation of selenophosphate through the activity of selenophosphate synthetase (Tamura et al., 2004) and then incorporation of Selenophosphate into selenocysteyl-tRNA^{[Ser][Sec]} (Allmang et al., 2009). Se is excreted through the intermediate Selenide. At adequate level of intake it is incorporated into selenosugar A and B for excretion in urine through the activity of methyltransferase and SAM (Kobayashi, et al., 2002). At higher levels of intake the methyltransferase add the methyl group from SAM to convert selenide to methylselenol then dimethylselenide, then trimethylselenonium, which is excreted in the urine (Ohta and Suzuki, 2008, Krittaphol, 2010). The methylselenol can be reduced to methyl-selenic acid through a reversible reaction with the thioredoxin reductase (Moore et al., 1964; Speranza et al., 1973; Arner and Holmgren, 2000)

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