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RESPONSES OF BOVINE PITUITARY TRANSCRIPTOME PROFILES TO CONSUMPTION OF TOXIC TALL FESCUE AND FORMS OF SELENIUM IN VITAMIN-MINERAL MIXES

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RESPONSES OF BOVINE PITUITARY TRANSCRIPTOME PROFILES TO CONSUMPTION OF TOXIC TALL FESCUE AND FORMS OF SELENIUM IN VITAMIN-MINERAL MIXES

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture, Food and Environment
at the University of Kentucky

By
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2019

ABSTRACT OF DISSERTATION

RESPONSES OF BOVINE PITUITARY TRANSCRIPTOME PROFILES TO CONSUMPTION OF TOXIC TALL FESCUE AND FORMS OF SELENIUM IN VITAMIN-MINERAL MIXES

The first goal of the current research was to determine whether gene expression profiles differed between whole pituitaries of growing beef steers grazing pastures containing high (HE) or low (LE) amounts of toxic endophyte-infected tall fescue. The global (microarray analysis) and selected targeted (RT-PCR) mRNA expression patterns of pituitaries collected from beef steers (BW = 266 ± 15.5 kg) that had been randomly assigned to undergo summer-long grazing (89 to 105 d) of either HE (0.52 ppm ergot alkaloids) or LE (< 0.03 ppm ergot alkaloids) pastures were compared. Gene expression data were subjected to one-way ANOVA. The pituitaries of HE steers had 542 differentially expressed genes, and the pattern of altered gene expression was dependent on treatment. Targeted RT-PCR analysis corroborated these findings, including decreased expression of DRD2, PRL, POU1F1, GAL, and VIP and that of POMC and PCSK1, respectively. Canonical pathway analysis (Integrated Pathway Analysis, IPA) identified HE-dependent alteration in signaling of additional pituitary-derived hormones, including growth hormone and GnRH. In conclusion, consumption of endophyte-infected tall fescue alters the pituitary transcriptome profiles of steers in a manner consistent with their negatively affected physiological parameters. The second goal of this project was to test the hypothesis that sodium selenite (ISe), SEL-PLEX (OSe), vs. a 1:1 blend (MIX) of ISe and OSe in a basal vitamin-mineral (VM) mix would differentially alter pituitary transcriptome profiles in growing beef steers (BW = 183 ± 34 kg) commonly grazing an endophyte-infected tall fescue (HE) pasture. Steers were randomly selected from herds of fall-calving cows grazing HE pasture and consuming VM mixes that contained 35 ppm Se as either ISe, OSe, or MIX forms. Steers were weaned, depleted of Se for 98 d, and subjected to summer-long common grazing of a 10.1 ha HE pasture containing 0.51 ppm ergot alkaloids. Steers were assigned (n = 8) to the same Se-form treatments on which they were raised. Selenium treatments were administered by daily top-dressing 85 g of VM mix onto 0.23 kg soyhulls, using in-pasture Calan gates. Pituitaries were collected at slaughter and changes in global (microarray) and selected (RT-PCR) mRNA expression patterns determined. The effects of Se treatment on relative gene expression were subjected to one-way ANOVA. The form of Se affected the expression of 542 annotated genes. Integrated

Pathway Analysis found a canonical pathway network between prolactin and POMC/ACTH/ α-MSH synthesis-related proteins, and that mitochondrial dysfunction was a top-affected canonical pathway. Targeted RT-PCR analysis found that the relative abundance of mRNA encoding prolactin and POMC/ACTH/ α-MSH synthesis-related proteins was affected by the form of Se, as were mitochondrial dysfunction-related proteins OSe steers appeared to have a greater prolactin synthesis capacity vs. ISe steers through decreased dopamine receptor D2 signaling, whereas MIX steers had a greater prolactin synthesis capacity and release potential by increasing TRH concentrations than ISe steers. OSe steers also had a greater ACTH and α-MSH synthesis potential than ISe steers. We conclude that form of Se in VM mixes affected genes responsible for prolactin and POMC/ACTH/α-MSH synthesis, and mitochondrial function in pituitaries of growing beef steers commonly grazing an HE pasture. The third goal was to test the hypothesis that sodium selenite (ISe), SEL-PLEX (OSe), vs. a 1:1 blend (MIX) of ISe and OSe in a basal vitamin-mineral (VM) mix would differentially alter selenoprotein profiles in pituitaries and livers of growing beef steers commonly grazing an endophyte-infected tall fescue (HE) pasture (i.e., the same steers used in Goal 2). The effects of Se treatment on relative gene expression were subjected to one-way ANOVA. The mRNA content of 6 selenoproteins in the pituitary was affected by Se treatments, along with two selenoprotein P receptors, whereas the expression of two selenoproteins was altered in the liver. We conclude that the change in selenoprotein gene expression in pituitaries indicates that OSe steers have a greater potential capacity to manage against oxidative damage, maintain cellular redox balance, and have a better quality control of protein-folding in their pituitaries than ISe steers. The change in selenoprotein gene expression by the liver indicates that MIX steers have a greater redox signaling capacity and capacity to manage oxidative damage than ISe steers.

KEYWORDS: Bovine pituitary, Ergot alkaloid, Fescue toxicosis, Prolactin, Selenium, Selenoprotein

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CHAPTER 1. Introduction

Epichloe coenophialum (formerly *Neotyphodium coenophialum*) is an endophytic fungus that infects most tall fescue (*Lolium arundinaceum*) pastures commonly used in animal grazing systems in the eastern half of the United States (Aiken and Strickland, 2013). The interaction between *E. coenophialum* and tall fescue produces ergot alkaloids (Siegel and Bush, 1994). Consumption of ergot alkaloid-containing tall fescue impairs several metabolic, vascular, growth, and reproductive processes in cattle, collectively producing a clinical condition known as "fescue toxicosis" (Strickland et al., 2011). There are over 8.5 million cattle grazing endophyte-infected tall fescue pasture (Paterson et al., 1995), and that causes approximately one billion dollar losses to beef industry in the United States annually (Oliver, 1997). Reduced serum prolactin is a recognized marker of fescue toxicosis (Goetsch et al., 1987; Davenport et al., 1993). The anterior pituitary gland secretes hormones that affect control over several physiological processes altered by consumption of ergot alkaloid-containing forages, including hormones for metabolism (TSH), growth (GH), reproduction (LH, FSH), stress responses (ACTH), and lactation (prolactin) (Beardwell and Robertson, 1981). Despite these known relationships, reports that describe the effect of fescue toxicosis on pituitary genomic expression profiles are still very limited.

Besides fescue toxicosis, another challenge faced by many southeastern United States cattle producers is selenium (Se) deficiency. Se-poor soils in this same geographic region result in Se-deficient forages necessitating the need to provide supplemental Se (Dargatz and Ross, 1996). Inorganic Se (ISe, sodium selenite) is the most common form of Se supplemented in cattle diets, whereas organic forms of Se (OSe) derived from

specially cultivated *Saccharomyces cerevisiae* also are available and approved for use in beef cattle diets. Serendipitously, it was found that expression of several genes downregulated in the liver (Liao et al., 2015) of steers grazing high vs. low endophyte-infected forages were upregulated in cattle by consumption of a 1:1 blend of ISe:OSe (MIX) in vitamin-mineral (VM) mixes (Matthews and Bridges, 2014; Matthews et al., 2014). Moreover, it was determined subsequently that steers subjected to summer-long grazing of endophyte-infected pasture and supplemented (3 mg/d) with MIX or OSe forms of Se had greater serum prolactin concentrations than ISe-supplemented steers (Jia et al., 2018). Hence, forms of Se seems to impact gene expression in steer pituitaries, specially genes associated with prolactin synthesis or secretion. However, the effects of forms of Se on gene expression profile in bovine pituitary has not been reported.

In addition, to our knowledge, studies regarding the effects of forms of Se on selenoprotein profile in bovine pituitary and liver tissues have not been reported either. The goals of this dissertation are stated in Chapter 3.

CHAPTER 2. Literature Review

2.1 Fescue toxicosis

The perennial tall fescue (*Lolium arundinaceum*, formerly *Festuca arundinacea*) is an economically important cool-season bunch-grass, originally from Europe (Buckner et al., 1979). It was first introduced to the United States from Eurasia in the late 1800s, but was not cultivated widely until the 1940s and 1950s (Hoveland, 2009). It is well adapted and the most widely grown grass in the transition zone between the northern and southern regions of the eastern United States (Hemken et al., 1984; Paterson et al., 1995). Today it is estimated that tall fescue covers more than 15 million ha; and in the southeastern US, the most dominant cultivar is Kentucky-31 tall fescue (Buckner et al., 1979; Schmidt and Osborn, 1993; McCulley et al., 2015).

Tall fescue possesses many desirable properties including ease and wide range of establishment and adaptation, extended grazing season, tolerance to adverse climate, soil, and poor management regimes, pest resistance, and high-yield seed production (Ball et al., 1987; Stuedemann and Hoveland, 1988; Roberts et al., 2009). Many of these properties has been attributed to its symbiotic relationship with a fungal endophyte (*Epichloe coenophialum*, formerly *Neotyphodium coenophialum*)(Clay, 1990; Glenn et al., 1996; Schardl et al., 2004), which resides within the leaf, sheaths, and flower culms of tall fescue (Porter and Thompson, 1992). The biochemical basis for endophyte-induced physiological changes in tall fescue is still a mystery, but association with endophytes has been reported to benefit tall fescue in growth, survival, and drought tolerance (Arachevaleta et al., 1989; Schardl et al., 2004).

Although endophyte-host relationship confers tall fescue with many desirable agronomic characteristics, grazing on endophyte-infected tall fescue usually reduces animal performance, especially for livestock like cattle, sheep, and horses (Porter and Thompson, 1992). There are over 8.5 million cattle grazing endophyte-infected tall fescue pasture (Paterson et al., 1995), and that causes approximately one billion dollar losses to beef industry in the United States annually (Oliver, 1997).

Generally, there are three syndromes occurring on cattle grazing tall fescue: fescue foot, fat necrosis, and fescue toxicosis. Fescue foot is a dry and gangrenous condition of feet, which usually occurred with colder environmental temperatures (Bush and Buckner, 1973). Rear feet are most commonly affected (Thompson and Stuedemann, 1993). The second syndrome fat necrosis is a condition of necrotic fat lesions surrounding the intestinal tract from the abomasum to the rectum (Bush and Buckner, 1973). Those two syndromes above are less common to occur and easy to recover when the injured cow is removed from consuming endophyte-infected toxic tall fescue (Bush and Buckner, 1973; Stuedemann and Hoveland, 1988). With regard to the last syndrome, consumption of endophyte-infected tall fescue impairs several metabolic, vascular, growth, and reproductive processes in cattle, collectively producing a clinical condition known as “fescue toxicosis”, also referred to as summer lump or summer fescue toxicosis (Strickland et al., 2011). Specifically, clinical signs of fescue toxicosis in cattle include decreased feed intake, BW gain, milk production, and reproductive efficiency, and elevated body temperature and respiration rates, rough hair coats and preference for shade (Strickland et al., 1993; Brown et al., 2009). This frequently occurring and widespread syndrome over tall fescue pasture regions is the major culprit responsible for

the economic loss in beef industry mentioned above. The reduction of serum prolactin is a recognized and the most commonly analyzed physiologic hallmark of fescue toxicosis (Goetsch et al., 1987; Davenport et al., 1993).

The symbiotic interaction between *E. coenophialum* and tall fescue yields ergot alkaloids (Siegel and Bush, 1994; Strickland et al., 2011). The distribution of ergot alkaloids within the tall fescue varies, with the highest concentration in seed heads (Rottinghaus et al., 1991). Unfortunately, ergot alkaloids are naturally occurring mycotoxins (Bush and Fannin, 2009), and consumption of ergot alkaloids is detrimental to animal productivity, especially for livestock like cattle, sheep, and horses (Porter and Thompson, 1992). Hence, ergot alkaloids have been reported to be responsible for the toxicity of endophyte-infected tall fescue (Lyons et al., 1986; Paterson et al., 1995; Guerre, 2015).

2.2 Ergot alkaloids

Ergot alkaloids are naturally occurring secondary metabolites produced by fungi including members of the *Claviceps* and *Neotyphodium* genera (Strickland et al., 2011). It was discovered that when administered appropriately, ergot alkaloids could be used to improve human health. In fact, the first isolated and identified ergot alkaloid was ergotamine, which was extracted from sclerotia of *Claviceps* for pharmaceutical use (Flieger et al., 1997). The beneficial and pharmacological effects of ergot alkaloids include: treatment of parkinsonism, migraine, thrombosis, cerebrovascular insufficiency, and stimulation of cerebral and peripheral metabolism, etc (Berde and Schild, 1978). However, for livestock producers, it is a totally different story. As mycotoxins, ergot

alkaloids significantly influence livestock health and productivity negatively around the world.

The ergotism in livestock is directly related to the tetracyclic ergoline ring among all ergot alkaloids (Figure 2.1), similar to the ring structure of the biogenic amines, dopamine, epinephrine, norepinephrine, and serotonin (Berde and Stürmer, 1978; Pertz and Eich, 1999). Based on the type of substituent on C-8 of the ergoline ring (Figure 2.1), ergot alkaloids can be divided into 3 classes: clavines, ergopeptines, and lysergic acid and derivatives (Figure 2.2). Among ergopeptines, ergovaline was predominant in endophyte-infected tall fescue pastures, accounting for over 80% of the total ergopeptines and 10 to 50% of the total ergot alkaloids (Lyons et al., 1986).

Ergot alkaloid exerts function through binding biogenic amine receptors, most of which belong to the G protein-coupled receptor family of transmembrane receptors (Goddard III and Abrol, 2007). In addition, ergot alkaloids can function as both agonists and antagonists as ligands (Berde, 1980) and bind with one or more receptor sites (Berde and Stürmer, 1978). Hence, a variety of biological problems are derived from the receptor-alkaloid interactions. The impact of consuming ergot alkaloids from endophyte-infected tall fescue on multiple physiological systems (cardiovascular, growth, reproduction) in livestock has been intensively reviewed (Strickland et al., 2011). One evident influence of ergot alkaloid exposure on the reproductive system is prolactin depression in sera (Hurley et al., 1980) and pituitary glands (Schillo et al., 1988). This symptom is relatively consistent than many others caused by consumption of ergot alkaloids (Strickland et al., 1993). Therefore, reduced prolactin has been adopted as a serological sign of ergot alkaloid exposure.

2.3 Prolactin

Bovine prolactin concentrations in plasma or serum are presented in Table 2.1.

2.3.1 Prolactin structure

Based on its genetic, structural, and biological properties, prolactin belongs to the cytokine superfamily which also includes hormones like growth hormone and placental lactogen (Horseman and Yu-Lee, 1994). The genes encoding these 3 hormones were derived from a common ancestral gene by duplication (Niall et al., 1971), which occurred approximately 400 million years ago (Cooke et al., 1981). In addition, the members of this family share a tertiary structure of 4 α helix and bind to a non-tyrosine kinase, single-pass transmembrane receptor (Trott et al., 2008).

The single human prolactin gene that consists of 5 exons and 4 introns is found on chromosome 6, with a gene size of 10 kb (Cooke et al., 1981). The transcription of prolactin is controlled by two independent promoter regions (Figure 2.3). The proximal promoter is required for pituitary-specific prolactin expression, while a superdistal promoter located 5.8 kb upstream of pituitary start site directs extrapituitary expression (Berwaer et al., 1991; Berwaer et al., 1994). The proximal promoter contains multiple binding sites for pituitary-specific positive transcription factor 1 (Pit-1, encoded by POU1F1) which plays a pivotal role in prolactin gene transcription and development of lactotrophs in the anterior pituitary (Fox et al., 1990; Gourdji and Laverriere, 1994). In contrast, the superdistal promoter drives extrapituitary gene expression in a Pit-1-independent manner (Gellersen et al., 1994).

Mature bovine prolactin contains a single chain of 199 amino acids with three intramolecular disulfide bridges between six cysteine residues, and it shares 80% amino

acid sequence homology with human prolactin (Wallis, 1974). Although the major form of pituitary-derived prolactin is about 23 kDa, other variants have been identified in human, as results of alternative splicing, proteolytic cleavage, and other post-translation modification (e.g. phosphorylation and glycosylation) (Freeman et al., 2000).

The predominant site for prolactin production is the anterior pituitary gland. Besides pituitary, prolactin has been reported to be expressed at much smaller amounts at multiple extrapituitary sites in human, including adipose tissue, brain, breast, endometrium, decidua, myometrium, immune cells, placenta, prostate, and skin (Ben-Jonathan et al., 1996; Fitzgerald and Dinan, 2008; Diakonova, 2014).

2.3.2 Prolactin receptor

Prolactin was given the name according to the fact that it promotes lactation in mammals (Riddle et al., 1933). However, as a very ancient hormone and that exists in all vertebrates, prolactin has been reported to possess over 300 separate biological activities (Bole-Feysot et al., 1998), more than activities of all other pituitary hormones combined (Fitzgerald and Dinan, 2008). Pituitary-derived prolactin acts as a classical circulating hormone, whereas extrapituitary prolactin is thought to function as a cytokine (Ben-Jonathan et al., 2007). Prolactin exerts its physiological function through interaction with its receptor.

The prolactin receptor is a single-pass membrane-bound protein, which belongs to the class 1 cytokine receptor superfamily (Bazan, 1990a, b). It is devoid of intrinsic tyrosine kinase activity and capable of phosphorylation by cytoplasmic proteins (Ben-Jonathan et al., 2007). Each prolactin receptor contains an extracellular region, a transmembrane region, and an intracellular region (Bole-Feysot et al., 1998). After the

extracellular domain of prolactin receptor binds to a ligand (e.g. prolactin), a ligand-induced prolactin receptor dimerization occurs which is obligatory for subsequent signal transmission (Bernichtein et al., 2010). The role of the transmembrane region in prolactin receptor activation and signaling transmission is still unclear. In contrast, the intracellular domain has been known as a crucial component in the initiation of prolactin receptor-mediated signal transduction (Freeman et al., 2000). After binding of prolactin to its receptor, several signaling pathways can be activated including the Janus kinase-signal transducer and activator of transcription (Jak-Stat), the mitogen-activated protein kinase (MAPK), and the phosphoinositide 3 kinase pathways (PI3K) (Clevenger et al., 2003), among which is the Jak-Stat cascade predominant pathway (Figure 2.4).

Prolactin receptor isoforms, due to alternative splicing during transcription, have been identified in different mammals. In mice, three major prolactin receptor isoforms have been described including short, intermediate, and long forms (Bole-Feysot et al., 1998). In human, six isoforms of prolactin receptor have been reported (Diakonova, 2014). However, only two distinct prolactin receptor isoforms have been identified in cattle based on their amino acid sequence. In addition, it has been suggested that only the long isoform can mediate the Jak-Stat pathway (Brym et al., 2005). Moreover, elevated mRNA expression of long isoform has been observed in prolactin-overexpressing breast cancer cells and prolactin-transgenic mice (Ling et al., 2000; Liby et al., 2003).

The prolactin receptor is ubiquitously expressed (Nagano and Kelly, 1994; Bakowska and Morrell, 1997; Bole-Feysot et al., 1998), which facilitates over 300 biological functions of prolactin on certain level. The murine studies have shown that the ratio of isoforms varies based on tissues, development stages, and reproductive stages

(e.g. estrous cycle, pregnancy, lactation) (Nagano and Kelly, 1994; Bole-Feysot et al., 1998).

2.3.3 Biological functions of prolactin

Although best known for the role in regulating lactation, prolactin affects a wide variety of biological functions including regulations of reproduction, osmoregulation, immune responses, metabolism, and growth and development. The biological functions of prolactin are summarized in Table 2.2.

2.3.4 Prolactin synthesis and secretion by the anterior pituitary

The pituitary is an endocrine gland composed of anterior, intermediate, and posterior lobes, with the anterior lobe occupying approximately 80% of the entire gland. An anatomic horizontal view of pituitary is shown in Figure 2.5. The anterior lobe is composed of five tropic cell types, which together secrete six hormones: corticotrophs (ACTH), gonadotrophs (FSH and LH), lactotrophs (prolactin), somatotrophs (GH), and thyrotrophs (TSH). Lactotrophs represent a dynamic population of cells (20-50% of the anterior pituitary cells) depending on the gender and physiological status of the animal (Freeman et al., 2000). In addition, lactotrophs exhibit functional heterogeneity with regard to their responsiveness to secretagogues (Boockfor and Frawley, 1987). Lactotrophs located in the outer zone of the anterior pituitary gland are more responsive to thyrotropin releasing hormone (TRH), whereas those located in the inner zone respond greater to dopamine (Boockfor and Frawley, 1987; Arita et al., 1991).

Lactotrophs inherently possess a large storage capacity and a high basal secretory activity for prolactin. Hence, unlike other pituitary hormones such as LH or ACTH whose secretion regulation is provided in the form of positive stimulus by hypothalamus,

pituitary prolactin secretion is under tonic and predominantly inhibitory regulation by hypothalamus (Ben-Jonathan, 1985). The most influential hypothalamic prolactin-inhibiting factor is dopamine.

2.3.5 Dopaminergic regulation of prolactin secretion

Dopamine or its agonists have been reported to exhibit potent ability to inhibit prolactin release both in vivo and in vitro (MacLeod, 1969; MacLeod et al., 1970; MacLeod, 1976). There are three hypothalamic dopaminergic neuronal systems including tuberoinfundibular (TIDA), tuberohypophysial (THDA), and periventricular (PHDA) dopaminergic neuronal population (Freeman et al., 2000; Ben-Jonathan and Hnasko, 2001), among which the TIDA, residing in the arcuate nucleus and projecting to the external zone of median eminence, provides the major dopaminergic input to the anterior pituitary (Leong et al., 1983; Kawano and Daikoku, 1987). Dopamine exerts its inhibitory ability of prolactin secretion via binding to a G protein-coupled receptor expressed on the cell membranes of lactotrophs (Ben-Jonathan and Hnasko, 2001). This receptor (DRD2) belongs to D_2 subclass of the dopamine receptor family (Caron et al., 1978; Meador-Woodruff et al., 1989). In rats, two dopamine type two receptors isoforms (long and short) have been identified due to alternative splicing, and the long isoform is the predominant in the pituitary (Guivarc'h et al., 1995). Activation of either isoform in rat lactotrophs leads to repression of prolactin gene (PRL) expression (McChesney et al., 1991).

Dopamine inhibits prolactin gene expression by suppression of adenylyl cyclase activity. Most studies on inhibitory control of dopamine over prolactin expression used rats as models. In both male and female rat anterior pituitary homogenates, dopamine

inhibited basal and vasoactive intestinal peptide-induced adenylate cyclase in a dose-dependent manner. A specific DRD2 antagonist, sulpiride, stereo-specifically antagonized the dopamine inhibition of pituitary adenylate cyclase (Enjalbert and Bockaert, 1983; Enjalbert et al., 1990). Moreover, dopamine agonist bromocriptine inhibited prolactin gene promoter activity by 70%, and the inhibition was selective in that other viral or cellular promoters were unresponsive to bromocriptine. In addition to PRL promoter, dopamine caused a 60% decrease in transcription activity of Pit-1 promoter, and part of the inhibition was mediated by the two cAMP response elements (Elsholtz et al., 1991). Those findings combined suggest that DRD2-mediated inhibition of gene expressions of prolactin and Pit-1 by dopamine function through a canonical cAMP/PKA pathways.

The inhibition of prolactin secretion by activation of DRD2 receptors has been reported to link to modification of several potassium and calcium channels (Israel et al., 1987; Lledo et al., 1991). By binding with dopamine receptor DRD2, dopamine excites potassium conductance and inactivates voltage-sensitive calcium channels. As results, plasma membrane hyperpolarization is induced, intracellular calcium concentration is reduced, and prolactin secretion from secretory granules is inhibited. With the application of patch-clamp recording and use of antibodies raised against specific G proteins, it has been shown that the coupling of DRD2 to decreased calcium currents is via Gαo, whereas the coupling of DRD2 to increased potassium currents is via Gαi (Lledo et al., 1992). Therefore, the inhibition of prolactin release may be due to a combined function of DRD2 coupling with Gαi which evokes membrane hyperpolarization by opening

inwardly-rectifying potassium channel (indirect reduction in calcium influx) and DRD2 coupling with Gαo inhibiting voltage-dependent calcium channels.

Dopamine may suppress prolactin secretion through inhibition of inositol phosphate metabolism. In bovine pituitary cell preparation, dopamine agonists inhibited inositol phosphate accumulation and was prevented by DRD2 specific antagonists, suggesting that this effect is mediated through DRD2 activation (Simmonds and Strange, 1985). Therefore, DRD2 may be negatively coupled with the phospholipase responsible for PIP2 hydrolysis, involved in the phosphatidylinositol signal pathway. Activation of either isoform of DRD2 inhibits adenylyl cyclase. However, only the short isoform is coupled to the phospholipase signaling pathway negatively (Caccavelli et al., 1992; Senogles, 2000).

Ergot alkaloids ingested with consumption of endophyte-infested tall fescue structurally resemble various biogenic amines such as dopamine (Strickland et al., 2011). It is widely accepted that these ergot amines can bind to dopamine receptor DRD2, stimulate the receptors, and reduce basal level prolactin production and secretion through mechanisms described above.

2.3.6 Prolactin short-loop feedback regulation

PRL controls its own secretion by regulating the dopaminergic neuron through a short loop feedback mechanism (Figure 2.6). By interaction with prolactin receptor (PRLR) localized on TIDA neurons, prolactin stimulates an increase in dopamine synthesis in a time- and concentration-dependent manner. Specifically, it is achieved by stimulating site-specific (ser-19, -31, and -40) phosphorylation of tyrosine hydroxylase which is the rating-limiting enzyme in dopamine synthesis (Ma et al., 2005). In addition,

prolactin also exhibits the ability to elevate tyrosine hydroxylase mRNA expression levels in the arcuate nucleus (Arbogast and Voogt, 1991).

2.3.7 Thyrotropin-releasing hormone regulation of prolactin secretion

Thyrotropin-releasing hormone (TRH) is produced by the paraventricular nuclei in hypothalamus that is originally known to stimulate the release of thyroid-stimulating hormone from the anterior pituitary (Schally et al., 1966). Subsequently, it has been shown that TRH receptor is expressed on both thyrotrophs and lactotrophs (Pfleger et al., 2004), and TRH stimulates prolactin release from lactotrophs in a dose-dependent manner both in vitro and in vivo (Bowers et al., 1971; Blake, 1974). Although the specific mechanism has not been fully elucidated, TRH was found to induce prolactin mRNA levels via activation of ERK signaling pathway with synergistic increase in intracellular Ca^{2+} (White and Bancroft, 1983; Kanasaki et al., 2002). In addition, TRH activates its G protein-coupled receptor and then membrane-bound phospholipase C signaling and calcium influx, which consecutively enhances Ca^{2+}-dependent exocytosis of prolactin (Sikdar et al., 1989; Fomina and Levitan, 1995, 1997).

2.3.8 Vasoactive intestinal peptide regulation of prolactin secretion

Besides TRH, another hypothalamic pro-secretory agent of lactotrophs is vasoactive intestinal peptide (VIP). It was originally found in porcine small intestine (Said and Mutt, 1970). Subsequently, VIP was found in hypothalamus and median eminence (Besson et al., 1979; Dalcik and Phelps, 1993). Similar to TRH, VIP has been reported to stimulate prolactin secretion both in vivo and in vitro (Kato et al., 1978; Ruberg et al., 1978; Shaar et al., 1979). But unlike TRH, VIP is also synthesized in the lactotrophs, and the autocrine function of local production of VIP is thought to maintain

elevated basal prolactin release (Gómez and Balsa, 2004). By binding to high-affinity receptor on lactotrophs, VIP induces intracellular cAMP accumulation and then PKA activation (Miyata et al., 1989; Shivers et al., 1991), with a delayed increased in calcium concentration observed in the process (Samson et al., 1980; Bjøro et al., 1987).

2.3.9 Galanin regulation of prolactin secretion

Galanin is also known to stimulate prolactin release. Although galanin is also produced in arcuate nucleus of hypothalamus which projects to median eminence (Bedecs et al., 1995), it is primarily produced by the lactotrophs of the anterior pituitary (O'halloran et al., 1990). Specially, galanin peptide is found to be colocalized with prolactin in secretory granules of anterior pituitary cells (Hyde et al., 1991). Studies have shown that galanin secretion from cultured anterior pituitary cells is inhibited by somatostatin and dopamine, and stimulated by TRH and estrogen (Hyde and Keller, 1991; Hammond et al., 1997). Galanin exerts its function by binding its G protein-coupled receptor, which is present in the anterior lobe (Wynick et al., 1993). Although the mechanism has not been clearly defined, galanin is found to stimulate both basal and TRH-induced prolactin secretion in rats (Ottlecz et al., 1988). In addition, galanin may directly stimulate prolactin expression and act as a lactotroph growth factor, particularly when exposure to estrogen is high (Wynick et al., 1998).

2.3.10 Autocrine autoregulation of prolactin secretion

As mentioned above, prolactin regulates its own secretion by interacting with dopaminergic neurons in the hypothalamus. It has been reported that prolactin receptor also exists on the membrane of lactotrophs in the pituitary gland (Chiu et al., 1992). In

addition, it has shown that prolactin can inhibit its own secretion in an autocrine manner in human and rat in vitro studies (Hosojima and Wyche, 1985; Bentley and Wallis, 1987).

Freeman et al. (2000) argued that since a great proportion of lactotrophs actively release VIP, galanin, and prolactin, it is very possible that VIP, galanin, and prolactin may also regulate prolactin secretion in a paracrine manner.

2.4 Biosynthesis of proopiomelanocortin, ACTH, and α-melanocyte-stimulating hormone

Previously, a study was conducted by our lab using an animal model of beef steers grazing high vs. low endophyte-infected tall fescue and consuming ad libitum amounts of inorganic selenium-containing vitamin mineral (VM) mix. We found that concentrations of prolactin in steers grazing high endophyte-infected tall fescue (HE) were only 10% of those of the steers grazing low endophyte-infected tall fescue (LE) (Brown et al., 2009). In addition, the liver tissues of the HE steers had increased amounts of mitochondrial mass, capacity for ATP synthesis, and amino acid-derived gluconeogenesis than those of the LE steers, and these processes have been implicated to be coordinated through the glucocorticoid receptor-mediated pathway (Liao et al., 2015). Since ACTH is the major hormone responsible for stimulation of the glucocorticoid biosynthesis and secretion by the cortex of adrenal glands (Simpson and Waterman, 1988; Stocco and Clark, 1996), the ACTH synthesis pathway might be affected in bovine pituitary by fescue toxicosis.

ACTH is synthesized within the anterior pituitary as part of a much larger precursor called proopiomelanocortin (POMC). POMC is mainly synthesized in the corticotrophs of the anterior pituitary and the melanotrophs of the intermediate pituitary,

respectively (Cawley et al., 2016). POMC in mammals consists of 3 exons, of which exons 2 and 3 are translated (Figure 2.7). It is post-translationally cleaved into smaller peptide hormones in a tissue-specific and cell-dependent manner by proprotein convertases. In the pituitary corticotrophs, proprotein convertase 1 (encoded by the PCSK1 gene) alone is expressed and cleaves POMC, producing the ACTH, β-endorphin, β-lipotrophin, amino-terminal peptide and joining peptide (Figure 2.7) (Millington, 2007).

ACTH also gives rise to a second peptide hormone, α-melanocyte-stimulating hormone (α-MSH) (Figure 2.7). Proprotein convertase 2 (encoded by the PCSK2) participates in production of α-MSH. In the intermediate pituitary, proprotein convertase 2 cleaves peptide products initially produced by proprotein convertase 1 into small bioactive peptides (Seidah, 2013). After cleavage of $ACTH_{1-39}$ into $ACTH_{1-17}$ by proprotein convertase 2, carboxypeptidase E (encoded by the CPE) processes $ACTH_{1-17}$ to α-MSH with the cooperation of peptidylglycine α-amidating monooxygenase 2 (encoded by the PAM) and an N-acetyltransferase (Kumar et al., 2016).

2.5 Selenium deficiency

The essential trace mineral selenium (Se) is of fundamental importance to animal health and performance. Bioavailable selenium in the forages affects animal health, depending on its deficiency or excess. Although it is harmful in excess, geographically, Se deficiency is a more severe problem to livestock production (Khanal and Knight, 2010). The Se content of forages is dependent on the Se type available and Se content in the soil of a certain region (Mehdi and Dufrasne, 2016). When soil Se content is smaller than 0.5 mg/kg (Hefnawy and Tórtora-Pérez, 2010) or plant Se content smaller than 0.1

mg/kg (National Academies of Sciences and Medicine, 2016), Se deficiency is considered. Unfortunately, besides fescue toxicosis, the other challenge faced by many south-eastern United States cattle producers is Se deficiency due to low soil and forage selenium content (Dargatz and Ross, 1996). Se deficiency causes a variety of negative effects on beef cattle production including increased incidence of early embryonic death, retained placentas, cystic ovaries and weak heat periods (Corah, 1996), reduced growth rate and immune responses (Cerny et al., 2016), white muscle disease, and diarrhea (National Academies of Sciences and Medicine, 2016).

2.6 Supplemental Se

Se-deficient forages necessitate the need to provide supplemental Se. Some of the Se supplementations include direct injections, salt licks and drenches, addition in drinking water, implants (Khanal and Knight, 2010; Mehdi and Dufrasne, 2016). The nutritional requirement of Se for both growing and finishing beef cattle is around 0.1 ppm (100 µg/kg) dry matter (DM) per day (National Academies of Sciences and Medicine, 2016). Both inorganic and organic forms of Se are approved by FDA for beef cattle production. Inorganic/mineral form of Se supplementation includes sodium selenate (SeO_4) and sodium selenite (SeO_3), and the latter is the most common form of Se supplemented in cattle diet. Organic form of Se supplementation includes selenocysteine (Sec) and selenomethionine (SeMet), both of which are the most common Se source in plants (Läuchli, 1993). They can also be produced from specially cultivated yeast Saccharomyces cerevisiae. This Se yeast, in which SeMet is predominant, is also approved for beef cattle production by FDA (Juniper et al., 2008).

2.6.1 Inorganic Se

Most selenium supplementation adopts inorganic form of selenium. Selenate and selenite are taken by plant and then enter into food chain. Both dietary selenate and selenite can be absorbed efficiently. The absorption rate for selenite is above 50%, while the absorption rate for selenate is nearly 91% (Van Dael et al., 2001). Renal excretion of selenate is more rapid and higher compared with selenite. Hence, despite the large differences in absorption and renal excretion between selenate and selenite, when provided at dietary intake dose, both Se compounds result in roughly equivalent bioavailability of selenium (Van Dael et al., 2001). After absorption, selenate has to be reduced to selenite so it can be further metabolized. Selenite can be reduced by TrxR or by reacting with glutathione (GSH) to selenide (Ganther, 1971; Kumar et al., 1992).

2.6.2 SeMet

SeMet, a methionine analogue with a Se atom instead of a sulfur atom, is the major selenocompound in plants. It is synthesized and incorporated into protein in place of methionine since tRNA for methionine recognizes SeMet as methionine (Schrauzer, 2000). After absorption via intestinal methionine transporter, dietary SeMet is either metabolized directly to reactive forms (selenide, then Sec) of Se via the transsulfuration pathway, mostly in liver, or randomly inserted into proteins in place of methionine (McConnell and Cho, 1967; Esaki et al., 1981; Wolffram et al., 1989). After SeMet-containing proteins degrade, SeMet is released to free methionine pool again (Figure 2.8). It has been reported that availability of Se from both dietary SeMet and tissue SeMet can be modulated by dietary methionine level (Waschulewski and Sunde, 1988). SeMet-

containing proteins serve as Se pool, especially when animal is exposed to a low-selenium environment for a limited time (Burk and Hill, 2015).

2.6.3 Sec

Sec is considered to be the 21st amino acid, which is a cysteine analogue with a Se atom instead of a sulfur atom. Due to different chemical properties between Se and sulfur, the p*Ka* of Sec is 5.2 while the p*Ka* of Cys is 8.25 (Huber and Criddle, 1967). Hence, at physiological pH Sec is more reactive than Cys, because the selenol group of Sec is in its ionized form, while the thiol group of Cys is mainly protonated and less reactive (Johansson et al., 2005). Sec is considered as the most biologically active form of Se, and most selenoprotein enzymes are involved in redox reactions because of their active Sec residue (Papp et al., 2007). In plants, Sec is involved in the reverse transsulfuration pathway as an intermediate in which SeMet is made (Sors et al., 2005). Compared to SeMet, it is less abundant in plant proteins (Olson et al., 1970).
Duo to its highly reactive properties, free Sec is methylated to Se-methylselenocysteine by some plants to detoxify it (Neuhierl et al., 1999). However, Se-methylselenocysteine is toxic to animals when they are exposed to it (Burk and Hill, 2015). In relation to absorption of dietary Sec, less is known compared to SeMet. However, studies have reported that selenocystine, oxidized form of Sec, can be taken up well by intestinal transporters for neutral and dibasic amino acids in cultured cells and competitively inhibit cystine absorption (Wolffram et al., 1989; Nickel et al., 2009).

2.7 Selenoprotein synthesis

Se exerts its biological function largely through the presence of Sec in selenoprotein. There are two unique features about selenoprotein mRNA than other

mRNA (Figure 2.9). One is a UGA codon in the open reading frame which encodes Sec. The UGA codon functions as both a codon for Sec biosynthesis and as a codon for protein synthesis termination in the nuclear genome (Leinfelder et al., 1988; Gladyshev and Hatfield, 1999). In mitochondrial, UGA also codes for tryptophan (Correa et al., 2014). The other feature is the presence of Sec insertion sequence (SECIS) element, a cis-acting stem-loop secondary structure downstream of the UGA codon, which locates in the 3' untranslated region (Low and Berry, 1996). The SECIS element is necessary for recognition of in-frame UGA, as a signal for Sec insertion (Berry et al., 1993). Eukaryotic SECIS elements share several properties, consisting of two helices separated by an internal loop, a non-Watson-Crick GA quartet, and an apical loop or bulge (Walczak et al., 1996; Labunskyy et al., 2014). The differences among SECIS elements dictates translation efficiency by affecting binding of trans-acting factors (Low et al., 2000; Latrèche et al., 2012).

There are three unique trans-acting factors for selenoprotein synthesis (Figure 2.9). Unlike other amino acid in eukaryotes, one interesting feature of Sec is that its biosynthesis occurs on its own tRNA, designated $tRNA^{[Ser]Sec}$ (Lee et al., 1989; Hatfield et al., 1994). $tRNA^{[Ser]Sec}$ is first aminoacylated with serine. This reaction is catalyzed by seryl-tRNA synthetase, and the newly formed seryl- $tRNA^{[Ser]Sec}$ become the backbone for biosynthesis of Sec (Lee et al., 1989; Leinfelder et al., 1989). Then seryl- $tRNA^{[Ser]Sec}$ is phosphorylated by O-phosphoseryl- $tRNA^{[Ser]Sec}$ kinase (Carlson et al., 2004). The newly formed intermediate phosphoseryl-$tRNA^{[Ser]Sec}$ serves as a substrate for Sec synthase to synthesize Sec, using monoselenophosphate as the Se donor which is generated by selenophosphate synthase 2 from selenide and ATP (Xu et al., 2006). The

other two trans-acting factors essential for selenoprotein synthesis in eukaryotes are SECIS binding protein 2 (SBP2) and the specific elongation factor EFsec. SBP2 is stably associated with SECIS elements and the ribosomes (Copeland et al., 2001). In addition, SBP2 also binds to EFsec (Copeland and Driscoll, 1999) which recruits selenocysteyl-tRNA$^{[Ser]Sec}$ and facilitates insertion of Sec into newly synthesized polypeptide (Tujebajeva et al., 2000).

2.8 Twenty-five selenoproteins

In recent years, it has been widely accepted that Se exerts its physiological functions through selenoproteins (Kim et al., 2011). There are 25 mammalian selenoproteins identified, in all of which Sec residues are present in the primary structure (Kryukov et al., 2003). Nearly all functionally characterized selenoproteins are redox enzymes in that Sec residues confer their catalytic redox activities (Papp et al., 2007). Selenoproteins have been classified according to their biological functions (1) antioxidant enzymes; (2) redox signaling; (3) Thyroid hormone metabolism; (4) Sec synthesis; (5) Se transportation and storage; (6) protein folding (7) unknown function (Figure 2.10).

Glutathione peroxidases

Glutathione peroxidase (GPx) was the first selenoprotein found (GPx1) in mammals (Flohe et al., 1973). There are eight known GPx homologs, five of which are selenoproteins (GPx1, GPx2, GPx3, GPx4, and GPx6). The other three GPx (GPx5, GPx7, and GPx8) homologs, and GPx6 homologs in some mammals (e.g. mouse), are not selenoenzymes because their active-sites contain Cys instead of Sec (Kryukov et al., 2003). The five GPx (selenoproteins) are present in different compartments of cell and have distinct characteristics (e.g. substrate specificity) (Brigelius-Flohé and Maiorino,

2013). Collectively, the antioxidant GPx family catalyzes hydrogen peroxide (H_2O_2) or hydroperoxides reduction to affect signaling, to defend against oxidative damage, and to maintain cellular redox homeostasis (Margis et al., 2008).

GPx1 is called classical or cytosolic glutathione peroxidase (also known as cGPx), although it can also be found in mitochondria (Brigelius-Flohé and Maiorino, 2013). It is the most abundant mammalian selenoprotein, and also one of the most thoroughly characterized selenoproteins. GPx1 is expressed in all cell types and most abundant in the liver and kidney. Structurally, mammalian GPx1 forms a homotetramer (Flohe et al., 1971; Awasthi et al., 1975). This cytosolic enzyme scavenges H_2O_2 and soluble organic hydroperoxides in a glutathione (GSH)-dependent manner (Rotruck et al., 1973; Gladyshev and Hatfield, 1999). It has been reported that H_2O_2 serves as an important signaling molecule, which is involved in regulation of various biological processes such as stress response, cell proliferation, and apoptosis (D'Autréaux and Toledano, 2007). Therefore, these biological processes are indirectly modulated by GPx1. Together with catalase and superoxide dismutase, GPx1 has been considered as one of the major antioxidant enzymes, protecting cells by reduction of toxic H_2O_2 (Lubos et al., 2011). GPx1 knockout mice are viable but more susceptible to oxidative stress (Fu et al., 1999). Hence, GPx1 seems to contribute to protection against oxidative stress, but is not indispensable. GPx1 is one of the most sensitive selenoproteins to Se deficiency. Dramatic decrease in GPx1 mRNA, protein, and activity has been observed in Se-deficient rats (Sunde et al., 1997), and it has been proposed that GPx1 may also functions as a Se buffer or storage protein (Sunde, 1994).

GPx2 is known as the gastrointestinal-specific enzyme (GI-GPx) as well. Structurally and functionally, GPx2 highly resembles GPx1. It is a tetramer consisting of identical 22-kd subunits like GPx1, and GPx2 is also capable of reduce H_2O_2 in cytosol using GSH. However, unlike ubiquitously expressed GPx1, GPx2 is preferentially expressed in the epithelium of gastrointestinal system although its mRNA can also be found in liver (Arthur, 2001; Brigelius-Flohé and Maiorino, 2013). About half of the total Se-containing GPx activity comes from GPx2 in gastrointestinal tract (Esworthy et al., 1998). Therefore, GPx2 plays an important role in defense against oxidative stress in this organ. In addition, it has been reported that GPx2 is important for protection of the gastrointestinal tract against inflammation and involved in cancer development, although the question that it is preventing or promoting carcinogenesis remains open (Chu et al., 2004; Naiki-Ito et al., 2007; Banning et al., 2011).

GPx3 is a glycosylated protein and the only extracellular Se-containing GPx (also known as pGPx). It is mainly secreted from kidney, but also present in adipose tissue, thyroid colloid lumen, etc (Maeda et al., 1997; Köhrle, 2005). Like GPx1 and GPx2, GPx3 is a tetramer, but its substrate specificity is broader. Even though GPx3 is capable of reducing H_2O_2 in the presence of GSH, as an efficient antioxidant in plasma it utilizes thioredoxin or glutaredoxin as reductants when the concentration of plasma GSH is fairly low. In fact, it has been reported that thioredoxin and glutaredoxin are better electron donors for GPx3 (Björnstedt et al., 1994). The promoter of GPx3 is frequently hypermethylated in a variety of tumors, so epigenetic regulation of GPx3 appears to be important in development of these tumors (Lee et al., 2005; Lodygin et al., 2005). GPx3

is widely considered as an indicator of Se status because it accounts for over 20% of total plasma Se (Gandin et al., 2009).

GPx4, also known as PHGPx, is different from other Se-containing GPXs in that it can reduce phospholipid hydroperoxides that are associated with membrane function (Herbette et al., 2007). It utilizes protein thiols in addition to GSH as electron donors in mammalian cells (Ursini et al., 1999; Imai and Nakagawa, 2003). The lack of an internal loop in its monomeric structure enables GPx4 to interact with bulky phospholipid hydroperoxides (Sherrer et al., 2011). GPx4 is present in cytosolic (ubiquitously expressed), mitochondrial (testes), or nuclear form (testes) in a wide range of cell types and tissues, and is one of the most abundant selenoproteins (Brigelius-Flohe et al., 1994; Labunskyy et al., 2014). GPx4 is an essential antioxidant, and knockout of its gene is embryonic lethal (Yant et al., 2003). In addition, GPx4 plays a fundamental role in sperm maturation and male fertility. Disruption of mitochondrial GPx4 causes male infertility (Schneider et al., 2009). In contrast to GPx1, GPx4 levels are not sensitive to Se availability, and therefore it is considered as a housekeeping gene under Se deficiency condition (Bermano et al., 1995; Hara et al., 2001).

GPx6 is the last identified Sec-containing glutathione peroxidase, and its homologs in some mammals (e.g. mouse) are not selenoenzymes. It has been reported that its mRNA is only found in embryos and olfactory epithelium (Kryukov et al., 2003). So far, the knowledge about GPx6 is very limited. It is suggested that GPx6 may have a tetrameric structure and is capable of reducing H_2O_2 and some soluble low-molecular-weight hydroperoxides (Labunskyy et al., 2014).

Thioredoxin reductases

Thioredoxin is a class of small, ubiquitous redox proteins present in all living cells, which protect cells against oxidative stress and facilitate redox signaling by catalyzing dithiol-disulfide exchange reactions of other proteins. The general description of enzymatic resections of thioredoxin system is presented in Figure 2.11. Oxidized thioredoxin need to be reduced by thioredoxin reductase (TrxR) to exert its functions again (Arnér, 2009). TrxR is a central component of the thioredoxin system because it is the only identified enzyme to catalyze the reduction of oxidized thioredoxin, in a NAPDH-dependent manner (Mustacich and Powis, 2000). There are three known TrxRs in mammals, all of which are homodimers and belong to the family of selenoproteins (Labunskyy et al., 2014). Unlike most of the selenoproteins containing Sec in the N-terminal, each TrxR contains a Sec residue in the C-terminal, which plays an essential role for the enzymatic activity of TrxR (Gladyshev et al., 1996).

TrxR1 is primarily present in cytosol, and is the major protein disulfide reductase in mammalian cells. TrxR1 reduces cytosolic thioredoxin as the major substrate (Arnér and Holmgren, 2000). Therefore, many thioredoxin-dependent physiological processes are reliant on TrxR1 including defense against oxidative stress, regulation of gene transcription, apoptosis and so on (Arnér and Holmgren, 2000; Nordberg and Arner, 2001; Rundlöf and Arnér, 2004). In addition, TrxR1 can also exerts its biological impart by reducing certain redox-active enzymes such as ribonucleotide reductases and methionine sulfoxide reductases (Stubbe and Riggs-Gelasco, 1998; Stadtman et al., 2002). Moreover, various non-disulfide compounds like hydroperoxides and selenite can be reduced by TrxR1. Selenite can be reduced by TrxR1 to hydrogen selenide, which serves as the Se donor for Sec biosynthesis (Ganther, 1999).

TrxR2, also named as TR3, is located in mitochondria and expressed in various cell types (Rundlöf et al., 2001; Lillig and Holmgren, 2007). It catalyzes the reduction of mitochondrial thioredoxin and hence plays a role in regulation of mitochondrial redox processes and scavenging reactive oxygen species in mitochondria (Nalvarte et al., 2004). Studies suggest that TrxR2 may also be involved in regulation of cell signaling (Prasad et al., 2014) and cell proliferation (Kim et al., 2003).

TrxR3, also known as thioredoxin glutathione reductase (TGR), exists primarily in testes (Arnér, 2009). Besides an FAD-binding domain, an NADPH-binding domain, and an interface domain like TrxR1 and TrxR2 (Biterova et al., 2005), it also contains an additional glutaredoxin domain in the N-terminal, which allows its glutathione and glutaredoxin reductase activity (Sun et al., 1999; Sun et al., 2001). Hence, this TrxR may be the bridge connecting thioredoxin and GSH systems. Due to its specific expression in testes, especially expressed at high level after puberty, TrxR3 is thought to play a role in sperm maturation (Su et al., 2005), although the most of its biological functions are still unknown.

Iodothyronine deiodinase

Another important selenoprotein family is thyroid hormone deiodinases, also designated iodothyronine deiodinases (DIOs). DIOs regulate activation and inactivation of thyroid hormones, which are essential for regulation of growth and development, thermogenesis, and energy expenditure (Mullur et al., 2014). The major thyroid hormone secreted by the thyroid gland is thyroxine (T4), which exhibits little intrinsic bioactivity and can be converted to biologically active 3,3’,5-triiodothyronine (T3) by reductive deiodination of T4 (Labunskyy et al., 2014). The metabolism of thyroid hormone by

DIOs is presented in Figure 2.12. There are three isoforms of DIOs, all of which form a homodimer structure and belong to Sec-containing membrane-bound enzymes (Lu and Holmgren, 2009). However, these DIOs occur in distinct subcellular locations and tissues. DIO1 and DIO3 are plasma membrane-bound enzymes, whereas DIO2 is located on the endoplasmic reticulum (Labunskyy et al., 2014). DIO1 is expressed primarily in liver, kidney, and thyroid; DIO2 in central nervous system, pituitary, thyroid, and brown adipose tissue; and DIO3 in vascular tissue, skin, and placenta (Mullur et al., 2014).

DIO1 converts the prohormone T_4 to active thyroid hormone T_3 by outer ring deiodination. In turn, DIO1 is also capable of converting T_3 and T_4 by inner ring deiodination to produce inactive T_2 and reverse T_3 (rT_3) (Bianco et al., 2002). Due to its ability to regulate thyroid hormone level in both ways, DIO1 was originally thought to be responsible for maintaining and regulating circulating levels of T_3 (Gladyshev and Hatfield, 1999). In contrast, DIO2 and DIO3 were proposed to regulate local intracellular T_3 level in a tissue-specific manner (Gereben et al., 2008a). However, it has been reported that DIO1 is dispensable for T_3 action in DIO1 gene-knockout mice (Schneider et al., 2006). In addition, recent studies show that DIO1 may be important to regulate high T_4 levels in hyperthyroid patients, but not in euthyroid human (Maia et al., 2005).

Currently, DIO2 is considered as the major producer of circulating T_3 in human (Maia et al., 2005). The activity of DIO2 can be elevated by low levels of serum T_4 (Gereben et al., 2008b). In addition, the intracellularly generated T_3 by DIO2 rather than DIO1 is more effective in regulation of gene transcription (Sagar et al., 2007). DIO2 also plays an important role in regulation of intracellular T_3 level in specific tissues. For example, in response to cold exposure, DIO2 regulates thermogenesis in brown adipose

tissue by elevating its expression level then increasing active T_3 level in this tissue without affecting circulating T_3 (Silva, 1995).

DIO3 inactivates T3 and T4 by deiodination of their inner ring in a tissue-specific manner. It is highly expressed in fetal and neonatal tissues and considered as the first expressed DIO developmentally (Mullur et al., 2014). It is expressed in highest level in placenta to prevent the access of maternal level thyroid hormones to a developing fetal tissues (Forhead and Fowden, 2014).

Selenoprotein P

Selenoprotein P (SelP) is an important selenoprotein in plasma of mammals. Unlike most other selenoproteins which usually contain one Sec residue, SelP contains 9-12 Sec residues per molecule in mammals (Read et al., 1990). For example, human SelP contains 10 Sec residues (Mangiapane et al., 2014). This unique property enables SelP to account for approximately 50% of the total Se in plasma (Burk and Hill, 2005). According to a Se supplementation study, SelP is a better maker for Se nutritional status than another important plasma selenoprotein GPX3 (Xia et al., 2005). Moreover, it has been suggested that the main role of SelP is in selenium transport, delivery, and storage (Burk et al., 1991; Saito and Takahashi, 2002). SelP is mainly synthesized in liver, although its mRNA can be found in all tissues (Labunskyy et al., 2014) and locally expressed SelP is proposed to play essential role in some tissues like brain (Schweizer et al., 2005). SelP is then secreted into plasma in a glycosylated form and delivered to remote tissues. After the degradation of SelP, its multiple Sec residues can be subsequently utilized to synthesize other selenoproteins (Richardson, 2005). In addition to selenium delivery and storage, studies have shown that SelP may also have heavy-

metal-chelator capacity (Seiler et al., 2008) and antioxidant functions (Takebe et al., 2002).

SelP is delivered to peripheral tissues through a receptor-mediated mechanism. There are two endocytic receptors from low-density lipoprotein receptor family, apolipoprotein E receptor-2 (apoER2) (Olson et al., 2007) and megalin (Olson et al., 2008). Both of these receptors have been identified as SelP receptors facilitating SelP entering into extrahepatic cells in a tissue-specific manner. ApoER2 is expressed highly in testis, bone marrow, placenta, brain, and muscle; moderately expressed in other tissues like thymus and spleen; and minimally expressed in liver and kidney (Burk and Hill, 2015). It is suggested the tissue hierarchy for Se in largely determined by SelP binding to apoER2 (Burk and Hill, 2015). The other SelP receptor megalin plays an essential role for uptake of plasma SelP in kidney, and the uptake is proposed to provide Se for GPx3 synthesis in kidney proximal tubules (Avissar et al., 1994). Megalin is also expressed in brain and reported to be involved in Se metabolism in brain (Chiu-Ugalde et al., 2010).

Selenophosphate synthetase 2

Selenophosphate synthetase 2 (SPS2) is homologous to selenophosphate synthetase 1 (SPS1) in mammals. However, unlike SPS1 in which the putative active center Sec is replaced by threonine, SPS2 belongs to the selenoprotein family (Low et al., 1995). Selenophosphate synthetase 2 (SPS2) catalyzes the conversion of selenide to active Se donor selenophosphate, which is required for Sec biosynthesis (Xu et al., 2007). SPS2 is an interesting selenoprotein in that it possibly regulates its own biosynthesis (Guimarães et al., 1996) and therefore it is thought to plays an auto-regulatory role in

selenoprotein synthesis (Kim et al., 1997). Although it is a necessary component for the Sec synthesis machinery, other aspects of SPS2 biological function are still unclear.

Selenoprotein W

Selenoprotein W (SelW) is one of the earliest identified selenoproteins in mammals. In addition, SelW is also one of the most abundant selenoproteins in mammals (Labunskyy et al., 2014). It is localized in mainly the cytosol and bound to the cell membrane in a small fraction (Yeh et al., 1995). SelW is ubiquitously expressed with the highest expression levels in muscles and brains (Gu et al., 2000). The expression of SelW is regulated by the availability of dietary Se (Howard et al., 2013). Its expression is downregulated in muscle, skin, prostate, heart, and intestine under Se deficiency while its expression in brain does not alter (Whanger, 2000). It has been proposed that SelW may play a role in redox-related process regulation because it binds GSH to form a complex with very high affinity (Beilstein et al., 1996). However, the molecular mechanisms involved remain to be elucidated.

15-kDa selenoprotein (Selenoprotein F)

The 15-kDa selenoprotein (Sep15) is one of the earliest identified selenoproteins. It is named by its molecular mass (Behne et al., 1997). A single Sec residue locates in the middle of the protein (Gladyshev et al., 1998). Sep15 is expressed in a wide range of tissues in mammals, with the highest levels in liver, kidney, prostate, and testis (Kumaraswamy et al., 2000). Its expression is sensitive to dietary Se intake (Ferguson et al., 2006). Sep15 has a thioredoxin-like domain, and N-terminal signal peptide, which is consistent with its location to the ER (Labunskyy et al., 2014). In addition, Sep15 interacts with the UDP-glucose: glycoprotein glycosyltransferases (UGGT) to form a

tight complex. UGGT is an ER-resident chaperone which is involved in N-linked glycoproteins folding in ER (Hebert et al., 1995; Molinari and Helenius, 1999). Hence, Sep15 is proposed to be involved in disulfide-bond formation and quality control of some glycoproteins in the ER (Labunskyy et al., 2007). Moreover, studies also suggest that Sep15 may be involved in cancer etiology in various types of tissues (Kumaraswamy et al., 2000; Hu et al., 2001; Apostolou et al., 2004; Nasr et al., 2004; Irons et al., 2010).

Selenoprotein H

Like most of the selenoproteins, selenoprotein H (SelH) contains a Sec residue with the CXXU motif at the N-terminal (Barage et al., 2018). Uniquely, SelH is found to reside specifically in nucleoli (Novoselov et al., 2007). Its expression is regulated by dietary Se (Howard et al., 2013). It contains a motif of DNA-binding proteins, which is consistent with its subcellular localization (Labunskyy et al., 2014). Furthermore, SelH is found to possess GPx activity, so it is proposed that SelH may be play a nucleolar oxidoreductase role (Novoselov et al., 2007).

Selenoprotein I

Selenoprotein I (SelI) can be only found in vertebrate and considered as a recently evolved selenoprotein (Kryukov et al., 2003). Structurally, SelI is similar to choline and choline/ethanolamine phosphotransferases except that SelI contains a Sec residue in the C-terminal region (Labunskyy et al., 2014). However, currently the function of SelI is still unknown.

Selenoprotein K

In contrast to SelI, selenoprotein K (SelK) is one of the most widespread selenoproteins, which exists in nearly all Se-utilizing organisms (Labunskyy et al., 2014).

SelK contains a Sec residue at the C-terminal end. It is expressed in a variety of tissues, with the highest expression levels in heart and skeletal muscle (Papp et al., 2007). This protein is localized to the ER membrane (Lu et al., 2006) and implicated in degradation of misfolded proteins (Shchedrina et al., 2011). In addition, the possible role of SelK in antioxidant function (Lu et al., 2006), anti-inflammation (Vunta et al., 2007) and immune response has been proposed (Misu et al., 2010).

Selenoprotein M

Selenoprotein M (SelM) is distantly related to Sep15 (31% sequence identity). Like Sep15, SelM is a thioredoxin-like protein, which is localized on ER (Labunskyy et al., 2014). The Sec residue locates in the N-terminal region as a part of a CXXU motif (Korotkov et al., 2002). SelM shows the highest level of expression in brain and thyroid, and moderate level in heart, kidney, lung, and placenta (Korotkov et al., 2002). The physiological function of SelM still remains unclear, but it is proposed to be involved in neuroprotection against oxidative damage by H_2O_2 and regulation of Ca^{2+} release from ER in neurons (Reeves et al., 2010).

Selenoprotein N

Selenoprotein N (SelN) contains the Sec residue in the C-terminal region (Kryukov et al., 2003). SelN is a transmembrane glycoprotein, existing in the ER membrane. It is ubiquitously expressed in tissues, but highest expressed during embryonic development (Petit et al., 2003). In adult tissues, it is highly expressed in muscle; and it has been reported that SelN clearly plays a vital role in maintenance of muscle progenitor satellite cells and regeneration of impaired skeletal muscle (Castets et al., 2011).

Selenoprotein O

Selenoprotein O (SelO) contains a Sec residue at the C-terminal (Kryukov et al., 2003). It is the largest mammalian selenoprotein and present in mitochondrial of various tissues (Han et al., 2014). Currently, information about structural and physiological characterization of selenoprotein O (SelO) is very limited, besides SelO is proposed to be involved in redox interaction through its CXXU motif (Han et al., 2014).

Selenoprotein R

Selenoprotein R (SelR), also known as methionine sulfoxide reductase B1(MSRB1) or selenoprotein X, is a zinc-containing stereospecific methionine R sulfoxide reductase. It is predominantly localized in the nucleus and cytosol (Kim and Gladyshev, 2004). SelR is sensitive to dietary Se intake (Novoselov et al., 2005). It is the major mammalian methionine sulfoxide reductase B with the highest activity in liver and kidney (Kim and Gladyshev, 2004). SelR catalyzes the specific reduction of R-isomer of oxidized methionine in proteins. Therefore, it is required for repair of oxidative damaged proteins (Kryukov et al., 2002). In addition, it is reported that SelR is important for regulation of cellular functions by reduction of methionine residues of regulatory proteins (Lee et al., 2013).

Selenoprotein S

Selenoprotein S (SelS) one of the most widespread selenoproteins. It contains a Sec residue at the C-terminal end (Kryukov et al., 2003). Like SelK, SelS is also localized to the ER membrane and implicated in degradation of misfolded proteins (Labunskyy et al., 2014). In addition, the possible roles of SelS includes regulation of inflammatory and immune response (Curran et al., 2005; Gao et al., 2006).

Selenoprotein T

Selenoprotein T (SelT) is a thioredoxin-like protein with a Sec residue located in the N-terminal region (Kryukov et al., 2003). It is primarily localized to ER membrane (Shchedrina et al., 2010). SelT is a ubiquitously expressed selenoprotein (Dikiy et al., 2007). It is highly expressed in embryonic tissues. In adult tissues, SelT maintains a high expression level in endocrine organs like pituitary, thyroid, and pancreas (Tanguy et al., 2011; Prevost et al., 2013). The biochemical function of SelT remains unclear. The proposed roles that SelT is involved in include: endocrine homeostasis, brain development and function, and neuroprotection (Youssef et al., 2018).

Selenoprotein V

Until now, selenoprotein V (SelV) is the least conserved mammalian and also the least characterized selenoproteins. It is only identified in testes of some placental mammals (Mariotti et al., 2012), and its function is still a mystery.

2.9 Effects of forms of dietary Se on circulating Se and prolactin concentrations, and gene expression profile in cattle

It has been reported that administration of organic Se leads to higher Se concentrations in biopsied liver tissue, jugular whole blood, and red blood cells compared to feeding the same doses of selenite in growing beef heifers (Liao et al., 2011). Later on, a 1:1 MIX (ISe:OSe) has been reported to be as potent as OSe in terms of elevating Se level of whole blood, red blood cells, serum, and liver in growing heifers, compared to dietary ISe (Brennan et al., 2011). In a beef cow/calf grazing study, cows supplemented with OSe had greater blood Se concentrations than cows consuming ISe or MIX. In addition, whole blood Se concentrations of OSe and MIX calves were greater than that of

ISe calves, and correlated to and affected by Se source from the dam (Patterson et al., 2013).

In agreement with previous studies, a most recent study shows that steers grazing endophyte-infected tall fescue with supplemental OSe and MIX in a basal vitamin-mineral mix had greater whole blood Se than steers consuming ISe. In the same study, steers with MIX or OSe forms of Se was found to have greater serum prolactin concentrations than ISe-supplemented steers (Jia et al., 2018).

In addition, forms of Se have been reported to influence gene expression profiles in bovine tissues, like the liver and testis (Liao et al., 2011; Matthews et al., 2014; Cerny et al., 2016). Serendipitously, it was found that expression of several genes downregulated in the liver of steers grazing high vs. low endophyte-infected forages were upregulated in cattle by consumption of a 1:1 blend of ISe:OSe (MIX) in vitamin-mineral mixes (Matthews and Bridges, 2014; Matthews et al., 2014; Liao et al., 2015).

In contrast, to our knowledge, the effect of consumption of endophyte-infected tall fescue on pituitary transcriptome profiles has not been reported. In addition, little is known regarding the effect of Se form on transcriptome profiles in bovine pituitary. Moreover, effects of forms of Se on selenoprotein profile in specific bovine tissue have not been reported either.

Table 2.1. Bovine prolactin concentration in plasma or serum.

Plasma/Serum	Species	Prolactin levels	Reference
Serum	Cow	99.6~129.3 ng/mL	(Ghorbani et al., 1991)
Plasma	Cow	5.3~7.9 ng/mL	(Browning et al., 1998)
Plasma	Heifer	2.78~3.37 ng/mL	(Pandey et al., 2017)
Serum	Heifer	25 ng/mL	(Jones et al., 2008)
Serum	Steer	50~750 ng/mL	(Trout and Schanbacher, 1990)
Serum	Cow	20 ng/mL	(Ahmadzadeh et al., 2006)
Serum	Steer	138.5-146.6 ng/mL	(Aiken et al., 2006)
Serum	Steer	18-125 ng/mL	(Fribourg et al., 1991)
Plasma	Cow	22-58 ng/mL	(Do Amaral et al., 2009)
Plasma	Steer	5.3-11.8 ng/mL	(Auchtung and Dahl, 2004)
Plasma	Cow	6-19 ng/mL	(Anchtung et al., 2005)
Plasma	Cow	14.4-25.2 ng/mL	(Bluett et al., 2003)
Serum	Steer	3.6-36 ng/mL	(Brown et al., 2009)
Plasma	Steer	16-20 ng/mL	(Browning et al., 1997)
Plasma	Cattle	145.9-156.2 ng/mL	(Browning et al., 1998)
Plasma	Steer	25-45 ng/mL	(Browning, 2000)
Serum	Cattle	15-85 ng/mL	(Campbell, 2014)
Serum	Steer	42.5-103.5 ng/mL	(Carter et al., 2010)
Serum	Cattle	60-230 ng/mL	(Drewnoski et al., 2009)
Serum	Cattle	1-13 ng/mL	(Hill et al., 2000)
Serum	Steer	2-24 ng/mL	(Kendall et al., 2003)
Plasma	Steer	8.83-18.03 ng/mL	(Matthews et al. 2005)
Serum	Cattle	1.7-2.3 ng/mL	(Merrill et al., 2007)

Table 2.2. Biological functions of prolactin in mammals. Adapted from Kelly et al. (1998) by removal of non-relevant data. For completeness, all the categories have been included whereas the underlined organ/target are thought to be especially relevant to this project.

Organ or Target	Effect
Water and electrolyte balance	
Kidney	↓ Na^+ and K^+ excretion
Sweat gland	↓Na^+ and Cl^- in sweat
Intestine	↑ Water and salt absorption
Uterus	↑Ca^{2+}, Na^+, K^+, Cl^- in flushings
Placenta	↓Fluid volume in amnion
Growth and development	
Body	↑Postnatal body growth
Skin	Proliferation of keratinocytes
	Hair loss for nest building
Hair follicle	Hair growth
Fetal lung	Maturation, surfactant production
Heart	↑Ornithine decarboxylase (ODC) activity
Liver	Hepatocyte proliferation
	↓Cytokine gene expression in Kupffer cells
	Induction of growth factors
Liver, kidney	DNA hypomethylation
Kidney	↑ODC activity
Intestine	↑Intestinal mucosa
	Growth and changes in metabolism
Muscle	Proliferation of vascular smooth
	Induction of growth-related genes
Adipocytes	Preadipocyte differentiation
Pancreas	Proliferation of β-cell
	Increased β-cell-to-cell communication
Adrenal	↑ODC activity
Pituitary	Proliferation of GH3 cells
Germ cells	↑Maturation
Gonads	↑Weight
	↑ODC activity
Prostate	Proliferation of human BPH epithelial cells
	↑Growth
	↑ODC activity
Seminal vesicle	↑Growth
Amnion	↑DNA synthesis and creatine kinase activity
Brain	Astrocyte proliferation

Table 2.2 (continued)

	Tuberoinfundibular hypothalamic development
Immune system	↑Thymus and spleen weights
	Proliferation of lymphocytes
Endocrinology and metabolism	
Brain	Tissue-specific modulation of ATPases
	Modification of enzyme activities
Fetal lung	↑Phospholipid synthesis
Liver	↑Lipoprotein lipase activity
	↑Bile secretion
	↑ Na^+ /taurocholate co-transport
	↑Glycogen phosphorylase a activity
	↑Ca^{2+} concentration
	↑$PGF_{2\alpha}$ and PGE
	↑IGF-I production
Pancreas	↑Insulin secretion
	↓Glucose threshold for insulin secretion
	↑Glucokinase and glucose transporter 2
Adrenal	↑Steroidogenesis
	↑Adrenal androgens (DHEA, DHEA-S)
	↑Cortisol, altosterone
	↑21-Hydroxylase activity
Skin	↑Steroidogenic enzyme (3βHSD)
Many tissues	↑PRLR number
Brain and behavior	
Brain, CNS	Maternal behavior
	Grooming behavior
	Hyperphagia
	Adaptive stress responses
	Induced analgesia mimicking
	Psychosomatic reaction
	↓Libido
	↑REM sleep
	Sleep-wake cycle
	↑Lordosis behavior
Hypothalamus, striatum	↑Dopamine turnover
Hypothalamus	Maturation of neonatal neuroendocrine system
	↓GnRH secretion
	↓Frequency and pulsatility of LH
	↑Electrical activity of the VMH neurons
	↑PKC activity
Retina	↓THR receptors
	↑Photoreceptor destruction

Table 2.2 (continued)

	Reproduction
Mammary gland	Lobuloalveolar growth
	↑Milk protein synthesis
	α-Casein
	β-Casein
	Whey acidic protein
	β-lactoglobulin
	Late lactation protein of marsupials
	↑Lactose synthesis
	Lactose synthetase
	↑Lipid metabolism
	Acetyl-CoA carboxylase
	Fatty acid synthase
	Malic enzyme
	Lipoprotein lipase
	↑IGF-I binding protein
	↑EGF
	↑I20-kDa protein
	↑Muc 1 (glycosylated mucin)
	↑PRL-inducible protein
	↑Parathyroid-like peptide
Ovary	Luteotropic and luteolytic actions
	Ovum maturation
	↓Folliculogenesis
	↓3β-HSD
	↓Aromatase
	Potentiate effects of LH on 3β-HSD
	↓Ovulation
	↓Plasmin generation in preovulatory follicles
Granulosa cells	↓Estrogen production
	↓P450 aromatase
	↑Use of extracellular lipoproteins
	↑Progesterone production
	↑LH receptors
	↑20α-OH progesterone
	↓Progestins
	↓Luteinization
	↓cAMP and steroidogenesis
	Counteract morphological effects of LH
	↑Progesterone in cocultures of splenic macrophages removed at proestrus
	↑DAG
	↑101_2-Macroglobulin
Oocytes	↑Development competence and maturation

Table 2.2 (continued)

Luteal cells	Luteotropic action
	Luteolysis in pregnancy
	Luteolysis during estrous cycle
	↓20α-HSD
	↑Progesterone
	Control of delayed implantation and steroidogenesis
	↓37-kDa protein
Uterus	↑Progesterone receptors and progesterone effects
	↑PGE_2, phospholipase A_2, prostaglandin G/H synthase
	↑Fluid loss
	↓Progesterone metabolism
	↑Estrogen receptors
	↓Myometrial contraction
	↑General secretory activity of endometrium
	↑Leucine aminopeptidase activity
	↑Uteroglobin production
	Promote blastocyst implantation
	↑Glucose amine synthetase activity
Leydig cells	Involved in maintenance of cell morphology
	↑LH receptors
	↑Aromatase activity
	↑Steroidogenesis and androgen production
Sertoli cells	↑FSH receptors
Germ cells	↑Total lipids
	↑Spermatocyte-spermatid conversion
Spermatozoa	↑Ca^{2+} binding and/or transport of ejaculated and epididymal spermatozoa
	Energy metabolism
	↑ATPase activity
	↑Fructose rate
	↑Glucose oxidation
	↓Zn^{2+} content
	Maintenance of mobility and attachment to oocyte
	Shortening optimal preincubation period to acquire capacitation
Epididymis	↑Glycogenolysis and hexophosphate enzymes
	↑Sialic acid
	↑β-galactosidase and α-mannosidase activities
	↑Lipids

Table 2.2 (continued)

	↓Glycoprotein metabolism
Seminal vesicle	↓Sperm fertilizing and mobility capacity
	Azoospermia
	↑Fluid lipids
	↑Lipogenesis
	↑Phosphomonoesterase and acid phosphatase
	↓Glycosylation
Prostate	↑Weight
	↑Nuclear uptake of DHT
	↑Androgen receptor
	Improvement in estrogen-induced inflammation
	↑Epithelia secretory function
	Monosaccharide formation
	Amino acid oxidation and transamination
	↑Ornithine decarboxylase
	↑Citric acid secretion
	↑Mitochondrial aspartate
	Citrate oxidation and m-asconitase
	↑Pyruvate dehydrogenase E1α
	↑Aspartate transporter
	↑C3 subunit of prostatein, probasin, RWB gene
	↑ IGF-I and IGF-I receptor
Immunoregulation and protection	
Spleen	↑Weight
Thymus	↑Weight
	↑Thymulin production
Submandibular gland	↑Immunostimulatory activity
Lymphocytes	↑Hormonal immunity
	↑Adjuvant arthritis response
	↑Cellular immunity
	↑Antibody formation to sheep RBC
	↑IgG and IgM antibodies
	Reverse hypophysectomy-induced anemia, leukopenia, and thrombocytopenia
	↑Proliferation
	↑IL-2 receptors
	↑EPO receptors
	↑PRL receptors
	↓Apoptosis
	↑IFN-γ
	↑Graft rejection
	↑*c-myc*

Table 2.2 (continued)

	↑DNA synthesis
	↑T cell engraftment
Nb2 cells	↑Proliferation
	↓Apoptosis
	IRF-1 gene induced
	c-myc gene induced
	c-fos gene induced
	ODC gene induced
	Hsp 70 gene induced
	β-Actin gene induced
	pim-1 gene induced
	gif-1 gene induced
	bcl-2 gene induced
	bax gene induced
	T-cell receptor γ-chain gene induced
	Cyclins D2 and D3 gene induced
	Cyclin E, *cdk2*, *cdk5*, E2F-1 gene induced
	Clone 15, nuclear movement protein gene induced
	GnRH gene induced
	GnRH receptor gene induced
	Jak2 protein activated
	Fyn protein activated
	Stat proteins activated
	Ras protein activated
	Raf protein activated
	Vav protein activated
	Grb2 protein activated
	Sos protein activated
	Shc protein activated
	MAP kinase protein activated
	Stathmin protein activated
	IRS-1 protein activated
	PTP-1D protein activated
	PKC protein activated
	Casein kinase II protein activated
	PTK protein activated
	PI3 kinase protein activated
	cbl protein activated
	S6 kinase protein activated
	G proteins activated
	PLC protein activated
	Amiloride-sensitive Na^+/H^+ exchange system protein activated
NK cell	↑Susceptibility of primary leukemia cells

Table 2.2 (continued)

Macrophages	↑Cytotoxic effects ↑DNA synthesis ↑Activation ↑Cytokine gene expression in Kupffer cells following hemorrhage ↓Monoblastic growth-synergy with IFN-γ ↑Superoxide anion responsible for killing pathogenic organisms ↑Nitric oxide and protection against bacterial infection
Polymorphonuclear cells	↓Direct and spontaneous migration
Thymic nurse cell complexes	Regulation of lymphocyte/epithelial cell adhesive interactions
Mammary gland	↑IgA-secreting plasma cells
Liver	Induction of coagulation factor XII

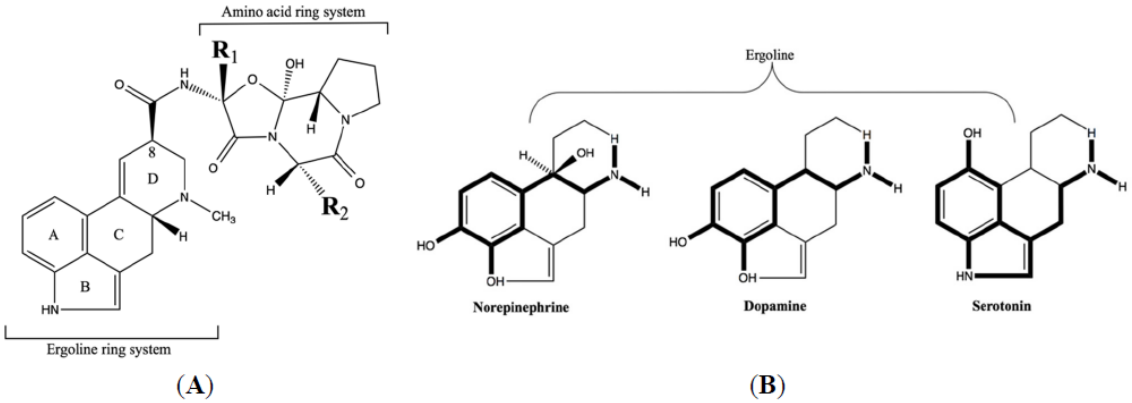

Figure 2.1. (A) The tetracyclic ergoline ring common to all ergot alkaloids that is variously substituted on the C-8 which in this case has an amino acid ring system that varies at the R1 and R2 substituents to create the various ergopeptine alkaloids. (B) The structural similarities between the ergoline ring and the catecholamines norepinephrine, dopamine, and serotonin (in bold) (Klotz, 2015).

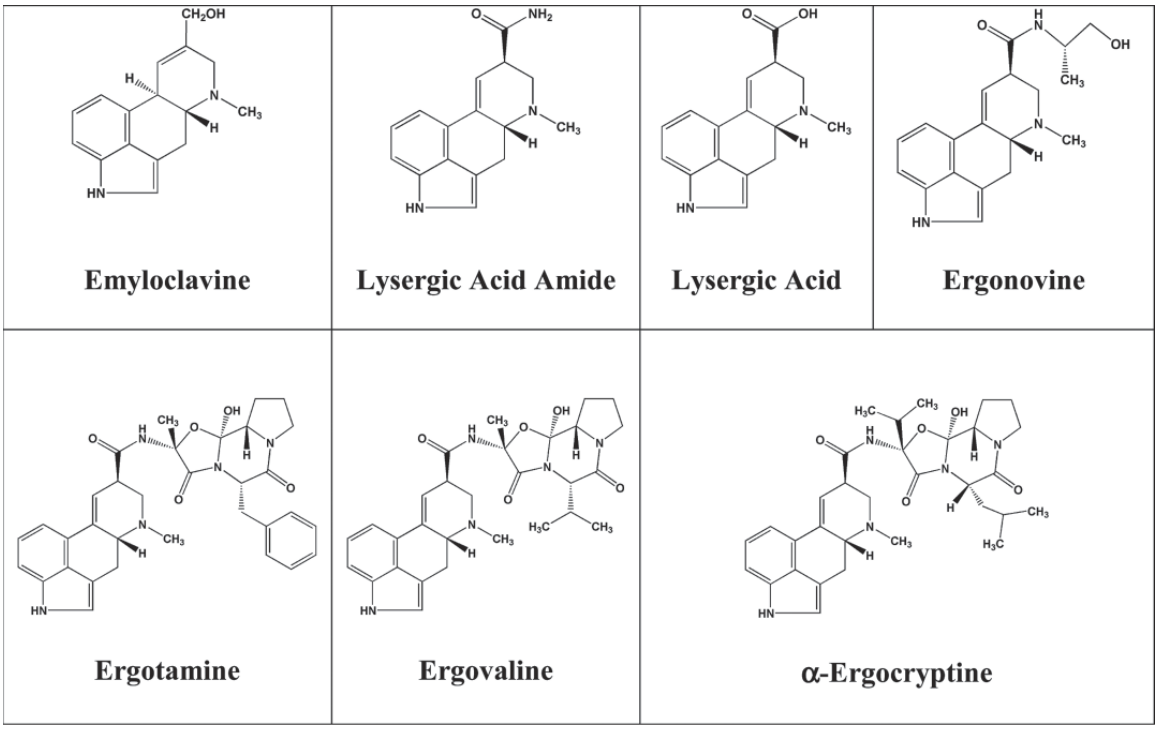

Figure 2.2 Chemical structures of the common ergoline ring structure, lysergic acid, and selected ergopeptines (Strickland et al., 2011).

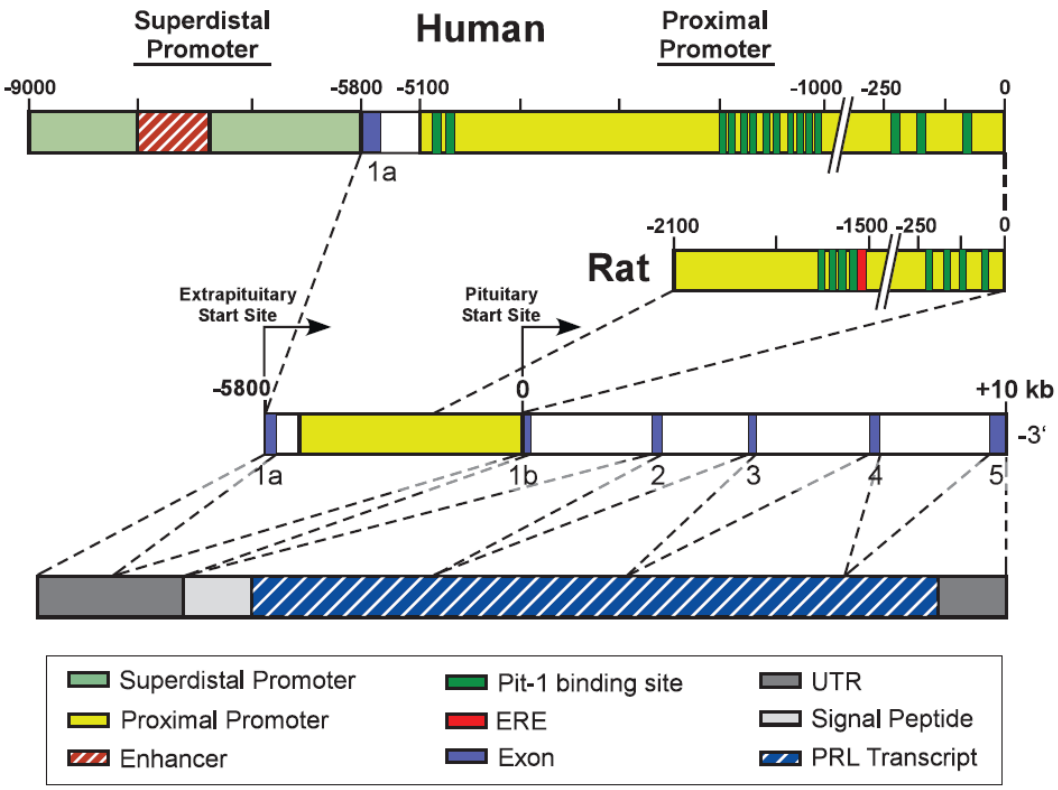

Figure 2.3. Diagram of the human and rat PRL promoters, the PRL gene, and the human mRNA transcript (Ben-Jonathan et al., 2007). Arrows designate transcriptional start sites for the proximal pituitary promoter and the superdistal extrapituitary promoter. The superdistal promoter is unique to humans, and its start site is located 5.8 kb upstream of the pituitary start site. The human and rat proximal promoters differ in size and contain 13 and 8 Pit-1 binding sites, respectively. A functional ERE is present in the rat promoter, whereas its presence in the human proximal promoter is questionable. In both species, the coding region in the pituitary consists of five exons that span approximately 10 kb. Transcription from either promoter produces mRNAs with identical protein coding sequences but differing in the 5' UTR. Due to the presence of an additional codon in the human gene (1a), extrapituitary PRL mRNA is about 150 bp longer than the pituitary

transcript. A signal peptide coding for 28-30 residues lies downstream of the UTR, followed by the PRL transcript.

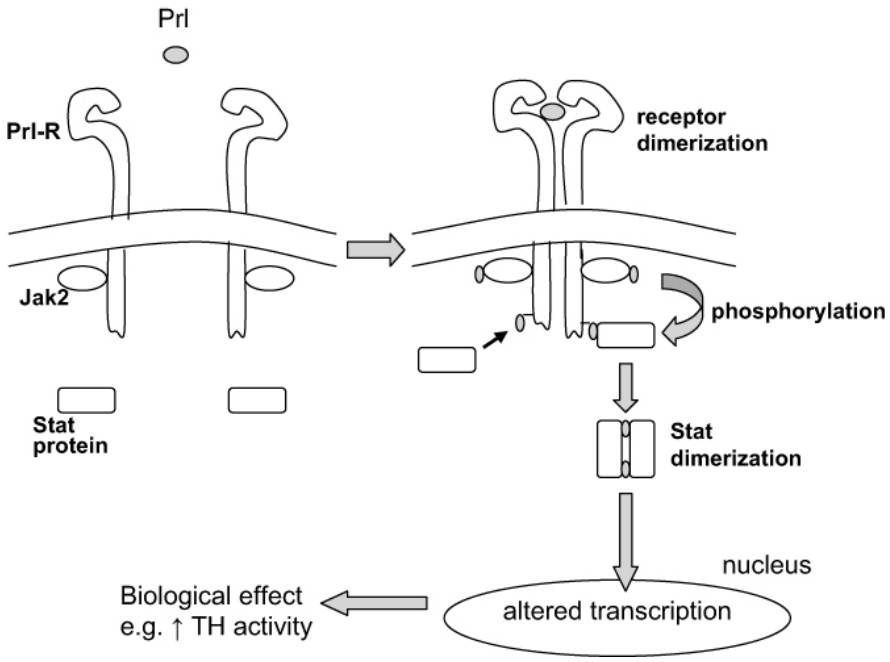

Figure 2.4. The predominant signal transduction pathway of prolactin (Fitzgerald and Dinan, 2008). Abbreviations: JAK, Janus kinase; Prl-R, prolactin receptor; STAT, signal transducer and transcription protein; TH, tyrosine hydroxylase.

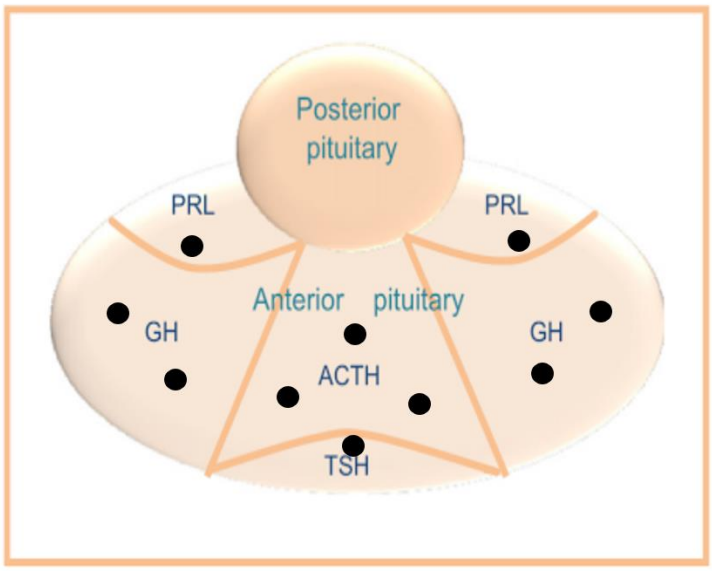

Figure 2.5. Horizontal view of pituitary emphasizing distribution and percentage of anterior pituitary cell subtypes. Adapted from Ben-Shlomo and Melmed, 2010. Luteinizing hormone (LH) - and follicle-stimulating hormone (FSH)-secreting cells (gonadotrophs) are scattered throughout the anterior pituitary and constitute about 10% of cells. PRL, prolactin-secreting cells (lactotrophs, 15%); GH, growth hormone-secreting cells (somatotrophs, 50%); ACTH, adrenocorticotropin-secreting cells (corticotrophs, 15%); TSH, thyroid-stimulating hormone-secreting cells (thyrotrophs, 5%).

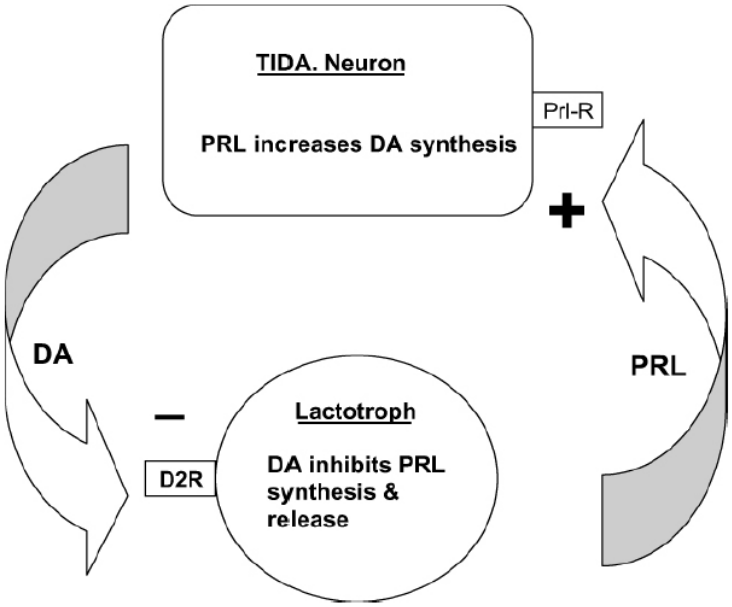

Figure 2.6. Short-loop feedback mechanism of prolactin regulation (Fitzgerald and Dinan, 2008). Abbreviations: DA, dopamine; TIDA, tuberoinfundibular dopaminergic neuron; Prl-R, prolactin receptor; D2R, dopamine2 receptor; PRL, prolactin.

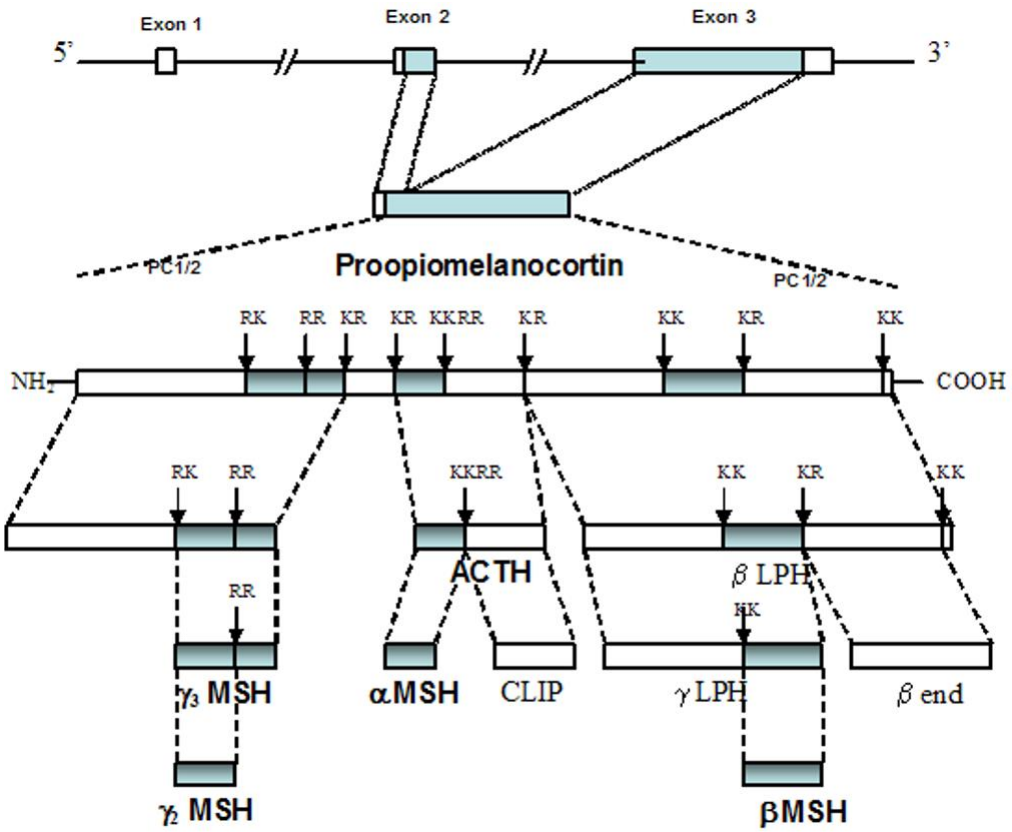

Figure 2.7. Gene structure and post-translational processing of POMC (Millington, 2007). Prohormone convertases 1 and 2 (PC1/2) break the parent POMC peptide into successively smaller peptides by cleavage at paired dibasic amino acid residues consisting of lysine (K) and/or arginine (R). The final products are generated in a tissue specific manner, for example α-MSH and ACTH are not produced by the same cells in the pituitary. They also involve additional enzymatic post translational modifications, such as the acetylation of α-MSH. The final products include the melanocortins (MSHs and ACTH), β-endorphin (β-end) and corticotrophin-like intermediate peptide (CLIP). There are intermediate peptides whose biological function remains unclear, such as β and γ lipotrophins (β-LPH, γ-LPH).

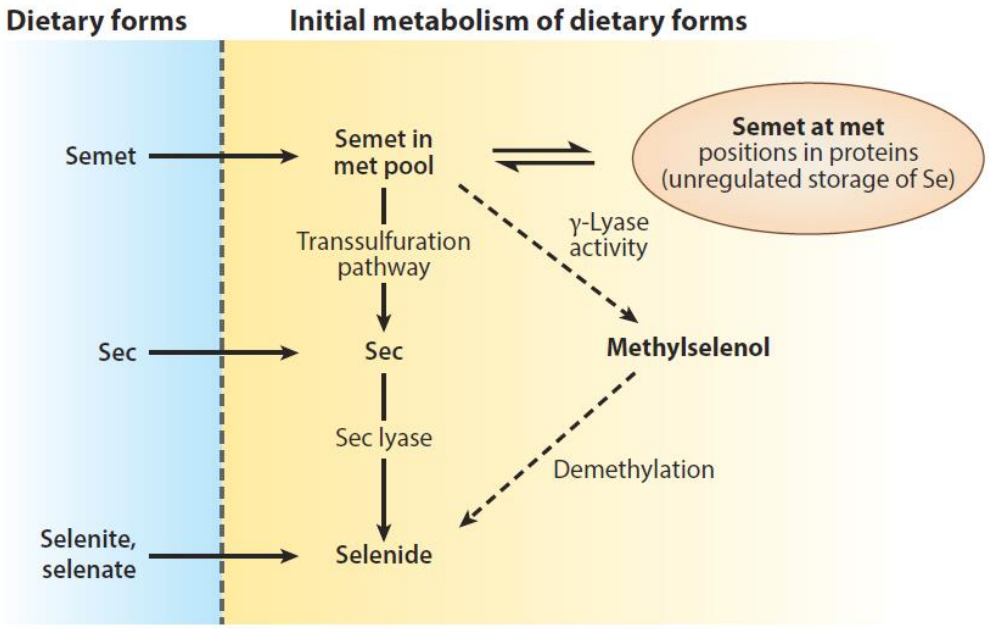

Figure 2.8. Initial metabolism of the principal dietary forms of selenium (Burk and Hill, 2015). Abbreviations: met, methionine; Sec, Sec; Semet, SeMet.

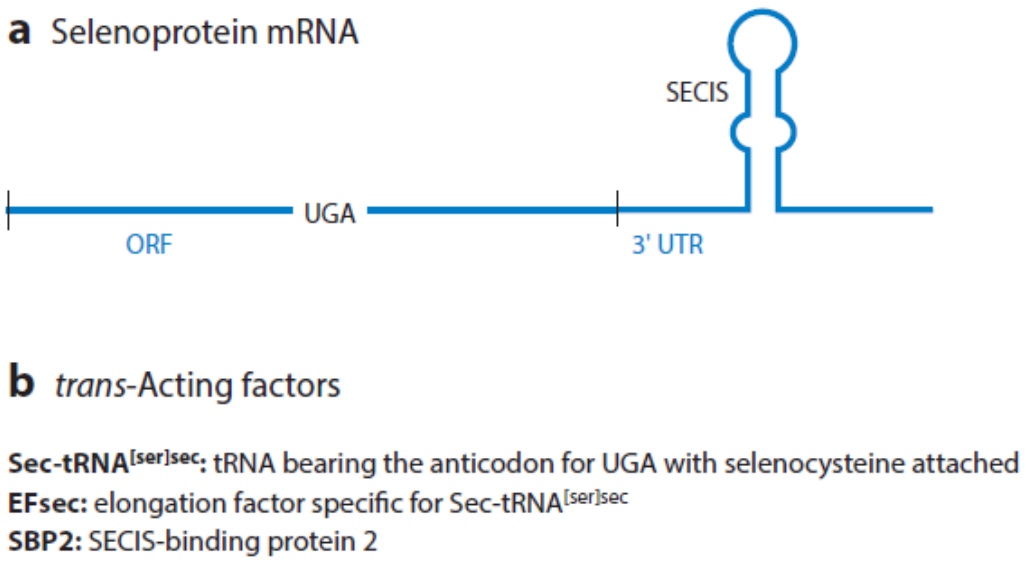

Figure 2.9. Factors essential for selenoprotein synthesis (Burk and Hill, 2015). Abbreviations: 3'UTR, 3' untranslated region; ORF, open reading frame; SECIS, selenocysteine insertion sequence element.

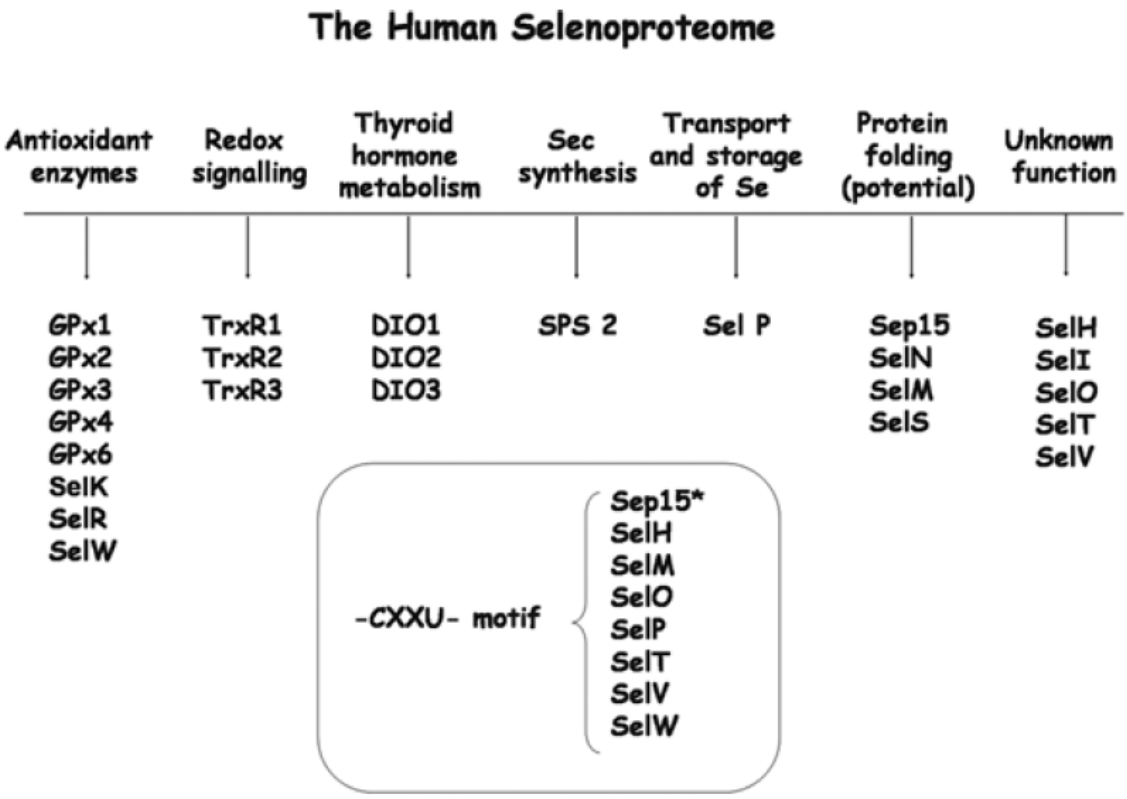

Figure 2.10. The human selenoproteome (Papp et al., 2007). The 25 human selenoproteins are outlined and classified by their determined or potential function. Selenoproteins with -CXXU- motifs are shown in the box. *-CXU- motif.

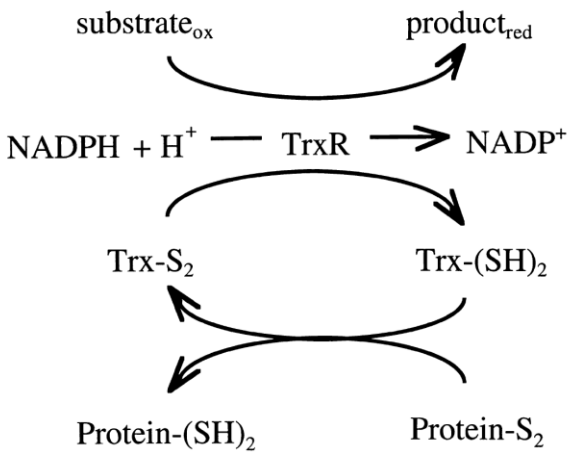

Figure 2.11. Scheme of oxidoreductase activities of the thioredoxin system (Arnér and Holmgren, 2000). The figure schematically depicts the reduction of the active site disulfide in oxidized thioredoxin, Trx-S_2, to a dithiol in reduced thioredoxin, Trx-$(SH)_2$, by thioredoxin reductase (TrxR) and NADPH.

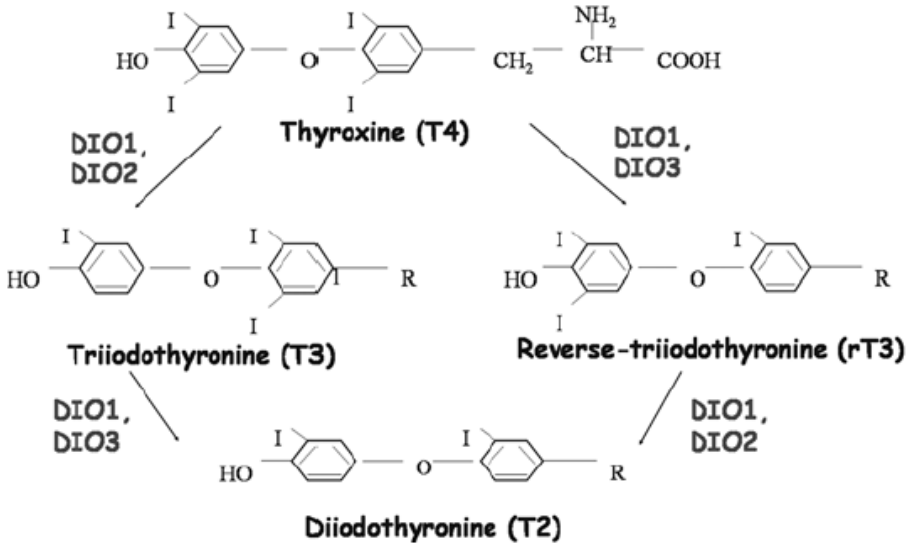

Figure 2.12. Metabolism of thyroid hormone thyroxine (T4) by the types 1, 2, and 3 deiodinases (DIO1, DIO2, DIO3) (Papp et al., 2007). DIO1 and DIO2 catalyze the reduction of T_4 to yield the active form of hormone T_3, or, reverse T_3, rT_3, to yield T_2. DIO1 and DIO3 catalyze the deiodination of T_4 to rT_3, and of T_3 to T_2.

CHAPTER 3. Dissertation Objectives

The goals of this dissertation were:

(1) to determine whether gene expression profiles differed between whole pituitaries of growing beef steers grazing pastures containing high (HE) or low (LE) amounts of toxic endophyte-infected tall fescue (Chapter 4).

(2) to determine whether the form of supplemental Se would alter pituitary transcriptome profiles of steers grazing endophyte-infected tall fescue (Chapter 5).

(3) to determine whether the form of supplemental Se would alter selenoprotein transcriptome profiles in pituitaries and livers of growing steers subjected to summer-long grazing of endophyte-infected pasture (Chapter 6).

The specific objectives of the dissertation were to answer the following questions:

(1) Does consumption of endophyte-infected tall fescue alter bovine pituitary transcriptome profiles? What canonical pathways are the differentially expressed genes involved in? Are there key upstream regulators associated with the differentially expressed genes? Does consumption of endophyte-infected tall fescue affect prolactin gene (PRL)? Does consumption of endophyte-infected tall fescue affect pituitary genes responsible for production and/or secretion of prolactin and/or ACTH? (Chapter 4)

(2) Does form of supplemental Se affect pituitary transcriptome of steers grazing endophyte-infected tall fescue? What canonical pathways are the differentially expressed genes involved in? Are there key upstream regulators associated with the differentially expressed genes? Does form of supplemental Se affect

prolactin gene (PRL)? Does form of supplemental Se affect pituitary genes responsible for production and/or secretion of prolactin and/or ACTH? Which form of supplemental Se could ameliorate the negative effect of consumption of endophyte-infected tall fescue in terms of elevating expression of genes associated with prolactin and ACTH production and/or secretion (Chapter 5)

(3) Does form of supplemental Se affect selenoprotein profiles in pituitaries and livers of steers grazing endophyte-infected tall fescue? Which tissue is more responsive? Are there selenoproteins tightly correlated with each other in terms of expression pattern, positively or negatively? Which form of supplemental Se benefits the steers the most based on their respective selenoprotein profile?

CHAPTER 4. Pituitary Genomic Expression Profiles of Steers Are Altered by Grazing of High (HE) vs. Low (LE) Endophyte-infected Tall Fescue Forages[1]

4.1 Abstract

Consumption of ergot alkaloid-containing tall fescue grass impairs several metabolic, vascular, growth, and reproductive processes in cattle, collectively producing a clinical condition known as "fescue toxicosis." Despite the apparent association between pituitary function and these physiological parameters, including depressed serum prolactin; no reports describe the effect of fescue toxicosis on pituitary genomic expression profiles. To identify candidate regulatory mechanisms, we compared the global and selected targeted mRNA expression patterns of pituitaries collected from beef steers that had been randomly assigned to undergo summer-long grazing (89 to 105 d) of a high-toxic endophyte-infected tall fescue pasture (HE; 0.746 μg/g ergot alkaloids; 5.7 ha; n = 10; BW = 267 ± 14.5 kg) or a low-toxic endophyte tall fescue-mixed pasture (LE; 0.023 μg/g ergot alkaloids; 5.7 ha; n = 9; BW = 266 ± 10.9 kg). As previously reported, in the HE steers, serum prolactin and body weights decreased and a potential for hepatic gluconeogenesis from amino acid-derived carbons increased. In this manuscript, we report that the pituitaries of HE steers had 542 differentially expressed genes ($P < 0.001$, false discovery rate ≤ 4.8%), and the pattern of altered gene expression was dependent ($P < 0.001$) on treatment. Integrated Pathway Analysis revealed that canonical pathways central to prolactin production, secretion, or signaling were affected, in addition to those related to corticotropin-releasing hormone signaling, melanocyte development, and pigmentation signaling. Targeted RT-PCR analysis corroborated these findings, including decreased ($P < 0.05$) expression of *DRD2*, *PRL*, *POU1F1*, *GAL*, and *VIP* and that of

POMC and *PCSK1*, respectively. Canonical pathway analysis identified HE-dependent alteration in signaling of additional pituitary-derived hormones, including growth hormone and GnRH. We conclude that consumption of endophyte-infected tall fescue alters the pituitary transcriptome profiles of steers in a manner consistent with their negatively affected physiological parameters.

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4.2 Introduction

Epichloe coenophialum is an endophytic fungus that infects most tall fescue (*Lolium arundinaceum*) pastures commonly used in animal grazing systems in the eastern half of the United States (Aiken and Strickland, 2013). The interaction between *E. coenophialum* and tall fescue produces ergot alkaloids (Siegel and Bush, 1994). Consumption of ergot alkaloid-containing tall fescue impairs several metabolic, vascular, growth, and reproductive processes in cattle, collectively producing a clinical condition known as "fescue toxicosis" (Strickland et al., 2011).

The anterior pituitary gland secretes hormones that affect control over several physiological processes altered by consumption of ergot alkaloid-containing forages, including hormones for metabolism (TSH), growth (GH), reproduction (LH, FSH), stress responses (ACTH), and lactation (prolactin) (Beardwell and Robertson, 1981). Despite these known relationships, we are unaware of reports that describe the effect of fescue toxicosis on pituitary genomic expression profiles.

The goal of the current research was to determine whether gene expression profiles differed between whole pituitaries of growing beef steers grazing pastures containing high (HE) or low (LE) amounts of toxic endophyte-infected tall fescue. We used transcriptome and targeted gene expression analyses to identify specific candidate molecules and signaling pathways responsible for the altered physiology of steers consuming HE forages. The global hypothesis tested was that consumption of endophyte-infected tall fescue would alter pituitary transcriptome profiles and that at least the pituitary genes responsible for the production and secretion of prolactin would be down-regulated and those for POMC/ACTH would be up-regulated.

4.3 Materials and methods

4.3.1 Animal model

All procedures involving animals were approved by the University of Kentucky Institutional Animal Care and Use Committee. The animal management regimen and model for steers that yielded the pituitary tissue of the present experiment have been reported. As described in detail previously (Brown et al., 2009; Matthews and Bridges, 2014; Liao et al., 2015), 19 beef steers (predominately Angus) were denied access to feed and water for 14 h, weighed, and subdivided into 2 groups based on BW. The steers were randomly allotted (d0) within BW group to graze either a low-toxic endophyte tall fescue-mixed pasture (LE; 5.7 ha; 0.023 μg ergot alkaloids/g; n = 9; BW = 267 ± 14.5 kg) or a high-toxic endophyte-infected tall fescue pasture (HE; 5.7 ha; 0.746 μg ergot alkaloids/g; n = 10; BW = 266 ± 10.9 kg) for an 89-d grazing period. Analysis of ergot alkaloid levels between the two pastures revealed that the HE steers were exposed to 25 and 21 times more ergovaline/ergovalinine and lysergic acid/isolysergic acid, respectively, than were the LE steers (Brown et al., 2009). After the common 89-d grazing period on pastures, steers were slaughtered in the University of Kentucky Meat Laboratory (Lexington, KY) over a 17-day period. Throughout the slaughter period, steers continued to graze their respective treatment pastures. Details of the slaughter period and process have been reported (Brown et al., 2009).

4.3.2 Sample collection and RNA preparation

Steers were stunned by captive bolt pistol and exsanguinated. Within 10 to 12 minutes of death, the whole pituitary was collected from each animal, placed in a foil pack, flash-frozen in liquid nitrogen, and stored at -80°C. Three pituitary glands (1 LE, 2

HE) were not used in the microarray analysis because of tissue damage incurred during the collection process. As a result, eight pituitaries (n = 8) for both LE and HE treatment groups were subjected to RNA analyses.

Total RNA was extracted from the whole frozen pituitary tissue using TRIzol Reagent (Invitrogen Corporation, Carlsbad, CA) following the manufacturer's instructions. The RNA concentrations were determined using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE), which revealed that all samples had an average concentration of 678 ng/μl and were of high purity with 260:280 nm absorbance ratios ranging from 1.71 to 1.91 and 260:230 nm absorbance ratios ranging from 2.08 to 2.55. The integrity of total RNA was examined by gel electrophoresis using an Agilent 2100 Bioanalyzer System (Agilent Technologies, Santa Clara, CA) at the University of Kentucky Microarray Core Facility. All RNA samples had 28S:18S rRNA absorbance ratios greater than 1.7 and RNA integrity numbers greater than 8.7.

4.3.3 Microarray analysis

The custom WT Btau 4.0 Array (version 1) GeneChip (Affymetrix, Inc., Santa Clara, CA) was used (Matthews et al., 2014) to investigate the effect of HE vs. LE consumption on bovine pituitary gene expression profiles. Microarray analysis was conducted according to the manufacturer's standard protocol at the University of Kentucky Microarray Core Facility. Briefly, 3 μg of RNA for each sample was first reverse-transcribed (RT) to cDNA and then from cDNA (double-stranded) to complementary RNA (cRNA; single-stranded), which was then labeled with biotin. The biotinylated cRNAs were further fragmented and used as probes to hybridize the gene chips in the GeneChip Hybridization Oven 640 (Affymetrix), using 1 chip per RNA

sample. After hybridization, the chips were washed and stained on a GeneChip Fluidics Station 450 (Affymetrix). The reaction image and signals were read with a GeneChip Scanner (GCS 3000, 7G; Affymetrix), and data were collected using the GeneChip Operating Software (**GCOS**, version 1.2; Affymetrix). The raw expression intensity values from the GCOS (i.e., 16 *.cel files from the raw methylation measurements) were imported into Partek Genomics Suite software (**PGS**, version 6.6; 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 chips and median polish summarization of multiple probes for each probe set.

All the GeneChip transcripts were annotated using the NetAffx annotation database for Gene Expression on Bovine GeneChip Array ST 1.1, provided by the manufacturer (http://www.affymetrix.com/analysis/index.affx, last accessed in March 2016, annotation file last updated in April 2014). Quality control of the microarray hybridization and data presentation was performed by MA plot on all the gene expression values and by box plot on the control probe sets on the Affymetrix chips (data not shown). Pearson (Linear) Correlation generated the similarity matrix (last accessed in March 2016, Partek Genomics Suite 6.6 6.15.0422). The average correlation between any pair of the 16 GeneChips was 0.98, and all GeneChips were further analyzed. Principal component analysis (**PCA**) was performed to elucidate the quality of the microarray hybridization and visualize the general data variation among the chips (Partek, 2015). To assess treatment effects (HE vs. LE) on the relative expression of the pituitary gene

transcripts, qualified microarray data were subjected to one-way ANOVA using the same PGS software. To achieve a higher degree of confidence (i.e., a more conservative approach), transcripts showing treatment effects at the significance level of $P < 0.001$ (false discovery rate of $\leq 4.8\%$) were defined as differentially expressed. These differentially expressed genes/gene transcripts (**DEGs**) were subjected to hierarchical clustering analysis using PGS software and to canonical, functional, and network pathway analyses using the Core Analysis program of Ingenuity Pathway Analysis online software (IPA, Build version 430059M, Content version 31813283; http://www.ingenuity.com [accessed in December, 2016]; Ingenuity Systems, Inc., Redwood City, CA).

All the microarray *.cel files collected by GCOS plus the GC Robust Multichip Averaging-corrected data processed by PGS software of this manuscript have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus (**GEO**; http://www.ncbi.nlm.nih.gov/geo/) [released October 23, 2014]), are minimum information about a microarray experiment (**MIAME**) compliant (Brazma et al., 2001), and are accessible through GEO series accession number GSE62570.

4.3.4 Real-time RT-PCR analysis

Primer sets for genes selected for real-time reverse transcription (RT) PCR analysis (Table 4.6) were designed using the NCBI Pick Primers online program against RefSeq sequences (accessed January to June 2016). Real-time RT-PCR was performed using an Eppendorf Mastercycler ep *realplex*2 system (Eppendorf, Hamburg, Germany) with iQ SYBR Green Supermix (Bio-RAD, Hercules, CA), as described (Bridges et al., 2012). Briefly, cDNA was synthesized using the SuperScript III 1st Strand Synthesis

System (Invitrogen), with 1 μg of RNA used for each reverse transcription reaction. Real-time RT-PCR was performed with a total volume of 25 μL per reaction, with each reaction containing 5 μL of cDNA, 1 μL of a 10 μM stock of each primer (forward and reverse), 12.5 μL of 2× SYBR Green PCR Master Mix, and 5.5 μL of nuclease-free water. Gene expression was analyzed by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

The resulting real-time RT-PCR products were purified using a PureLink Quick Gel Extraction Kit (Invitrogen) and sequenced at Eurofins Scientific (Eurofins, Louisville, KY). Sequences were compared with the corresponding RefSeq mRNA sequences used as the templates for primer set design. The sequences of the primers and the resulting sequence-validated real-time RT-PCR reaction amplicons for selected DEGs and the endogenous control genes *ACTB*, *PPIA*, and *UBC* are presented in Table 4.6 and Figure 4.2, respectively. Primers for *ACTB* were from Lisowski et al. (Lisowski et al., 2008), and primers for *s-PRLR* and *l-PRLR* were from Thompson et al. (Thompson et al., 2011). All sequenced amplicons had at least 98% identity with their template sequences. The raw CT values of *ACTB*, *PPIA*, and *UBC* in pituitary tissue of HE and LE steers did not differ (P = 0.57, 0.42, 0.82; respectively). Accordingly, the geometric mean expression of *ACTB*, *PPIA*, and *UBC* was used to normalize the relative quantities of the selected DEGs mRNA expression, and all RT-PCR reactions were conducted in triplicate.

4.3.5 Selected miRNA-target gene interactions

To identify (predict) microRNAs (miRNAs) that might regulate (Thompson et al., 2011; Agarwal et al., 2015) prolactin or POMC/ACTH production, microarray-identified

differentially expressed miRNAs (**DEMs**) were uploaded into TargetScan online software (Release 7.1, http://www.targetscan.org/), and the species-specific "Cow" filter applied. The resulting miRNA candidates were ranked based on cumulative weighted context++ scores (Agarwal et al., 2015) and then reduced to only those predicted to bind mRNA of genes involved in prolactin or POMC/ACTH production or to bind to mRNA coding known transcription factors of prolactin and POMC/ACTH pathway genes.

4.3.6 Statistical analyses

To test for HE vs. LE treatment effects on the relative expression of the pituitary gene transcripts, microarray hybridization data were subjected to one-way ANOVA using the PGS software as described in the "Microarray Analysis" section above. To determine the effect of treatment, the relative expression levels of selected DEGs analyzed by real-time RT-PCR were subjected to one-way ANOVA using the GLM procedure of the SAS statistical software package (version 9.4; SAS Inst., Inc., Cary, NC), with the endophyte level as the fixed effect. For these data, significance was declared when $P \leq 0.05$, and a tendency to differ was declared when $0.10 \geq P > 0.05$.

4.4 Results

4.4.1 Differentially expressed genes

Principal component analysis of all microarray data was performed to examine the correlation and variation among the chips, revealing a total variance of 30.9% (Figure 4.3). The first principal component (PC #1, x-axis) included genes with a median degree of variance (12.3%), whereas PC #2 (y-axis) and PC #3 (z-axis) encompassed genes that had low ranges of variance (9.84% and 8.75%, respectively). Overall, PCA clearly demonstrated that the chips within each treatment group were clustered closely together.

Individual ANOVA was conducted to identify altered expression of RNA transcripts in the pituitary tissue of HE vs. LE steers. At the $P < 0.01$ level and a false discovery rate of < 16%, 1,715 gene transcripts were identified. To refine this analysis, genes with the criteria of a false discovery rate of less than 4.8% and $P < 0.001$ were considered to be DEGs (Table 4.7). Of these 542 DEGs, 227 (10 non-annotated) were up-regulated, 5.5% to 79.8%, and 315 (14 non-annotated) were down-regulated, 5.7% to 69.0%, in HE vs. LE steers.

Hierarchical cluster analysis of the 542 DEGs revealed that all steers were clearly separated into either the LE or HE treatment group (Figure 4.4). Relative to LE steers, approximately 40% of the genes in the HE steers were up-regulated and 60% down-regulated.

4.4.2 Functional, canonical pathway, and gene network analyses

To determine the physiological significance of HE-induced DEGs (Table 4.7), bioinformatic analysis of canonical, functional, and network pathway analyses was performed. Canonical pathway analysis revealed ($P < 0.001$) that the top 7 pathways were the following: axonal guidance signaling (26 genes), role of NFAT in cardiac hypertrophy (16 genes), P2Y purigenic receptor signaling pathway (13 genes), cardiac hypertrophy signaling (17 genes), Tec kinase signaling (14 genes), ErbB signaling (10 genes), and CXCR4 signaling (13 genes) (Table 4.1). Additionally, several affected pathways central to prolactin production, secretion, or signaling were identified (Table 4.2), including dopamine receptor signaling, Gαi signaling, cAMP-mediated signaling, protein kinase A signaling, and prolactin signaling. Moreover, canonical pathway analysis also identified affected pathways involved in the signaling of other pituitary-

derived hormones (Table 4.3): corticotropin-releasing hormone signaling, melanocyte development and pigmentation signaling, growth hormone signaling, and GnRH signaling.

To refine this analysis to pituitary-specific metabolism, IPA analysis was re-run after applying the pituitary gland-specific filter. Diseases and Bio Function Analysis found ($P \leq 0.01$) putative changes in diseases and disorders, molecular and cellular functions, and physiological system development and function, resulting from the differential expression of 5 genes (*DRD2*, *PRL*, *ESR1*, *POMC*, and *TCF7L2*).

To gain insight into potentially interacting canonical pathways, pathway network analysis revealed one network that included 13 DEGs (*BTC*, *CPE*, *DRD2*, *ESR1*, *HAPLN1*, *IGF2*, *LAMA1*, *NCOA1*, *PCSK1*, *POMC*, *PRKCA*, *PRL*, and *REV3L*). Overlaying of canonical pathways revealed cross talk among several cell signaling pathways (Figure 4.1), including glucocorticoid receptor signaling (*ESR1*, *IL2*, *NCOA1*, *POMC*, *PRL*, *TGFB1*), GnRH signaling (*EGR1*, *FSHB*, *LHB*, *PRKCA*), growth hormone signaling (*CSHL1*, *IGF1*, *IGF2*, *PRKCA*), eNOS signaling (*ESR1*, *PRKCA*, *VEGFA*), dopamine receptor signaling (*DRD2*, *PRL*), and prolactin signaling (*PRKCA*, *PRL*).

4.4.3 Real-time reversed-transcribed PCR analysis of selected mRNA

Real-time RT-PCR analysis was used to corroborate the microarray analysis-identified altered expression of key genes responsible for prolactin synthesis and secretion and POMC/ACTH production in HE vs. LE steers (Table 4.4). The results of these two analyses were consistent for all the targeted genes, with the exception of *PRLR*, although the statistical significance (ANOVA P-value) and fold changes measured by the two analytical techniques differed for some genes. For *PRLR*, unlike the microarray

analysis, RT-PCR analysis was designed to delineate the long form (*l-PRLR*) and short form (*s-PRLR*). In the microarray analysis, *PRLR* was down-regulated in HE steers ($P < 0.001$), whereas in RT-PCR analysis, expression of *s-PRLR* was not altered ($P = 0.21$) and expression of *l-PRLR* had a tendency to differ ($P < 0.07$) in HE vs. LE steers.

Although microarray analysis did not identify them as DEGs (Table 4.8), the expression of 3 genes was assessed by RT-PCR analyses because they are known targets of POU1F1 (*GH1, TSHB*) or are involved in CRH stimulation of ACTH production (*CRHR1*). RT-PCR analysis corroborated the microarray analysis that pituitary expression of these genes did not differ between HE and LE steers (Table 4.4).

4.4.4 Differentially expressed miRNAs (DEMs) and their predicted target genes associated with prolactin and POMC/ACTH production

The microarray chips used for this study detected 574 miRNAs. Of these, only 6 were differentially expressed ($P < 0.001$) in HE vs. LE steers (Table 4.7). Specifically, miR-380 (42%), miR-2318 (17%), miR-329B (36%), and miR-544A (38%) were down-regulated in HE vs. LE steers, whereas miR-2356 (38%) and miR-2400 (8%) were up-regulated. The target genes of these DEMs that were associated with prolactin or POMC/ACTH production are listed in Table 4.5. Although no miRNAs known to directly target mRNA for prolactin were differentially expressed, every DEM targeted multiple prolactin transcription factors, stimulators, and (or) inhibitors, including miR-544A that targeted all the *PRL*-associated genes. Overall, the mRNA for three transcription factors (*POULF1*, *ESR1*, *PREB*), two transcription stimulators (*EGF*, *IKZF1*), and one transcription inhibitor (*PKIA*) of *PRL* were predicted to be targets of the DEMs. With

specific regard to microarray-identified DEGs (Table 4.4) targeted by DEMs, *PRLR* was predicted to be the target of five DEMs and *ESR1* the target of four DEMs.

Analogously, for POMC/ACTH production genes, whereas no miRNAs were differentially expressed that targeted *POMC* per se, TargetScan predicted that DEMs would interact with the mRNA of three transcription factors (*TBX19*, *NEUROD1*, *JUN*), two transcription stimulators (*LEP*, *LIF*), and three transcription inhibitors (*NR3C1*, *SMARCA4*, *HDAC2*) of the POMC production pathway. With specific regard to microarray-identified DEGs targeted by DEMs, *PCSK1* was the target of a single miRNA (miR-380).

Because *POMC* expression was altered and miR-380 is predicted to target two *POMC* transcription factors (*NEUROD1*, *TBX19*), the expression of *NEUROD 1* and *TBX19* was evaluated by RT-PCR, although their expression was not altered as determined by microarray analysis (Table 4.8). However, consistent with the microarray analysis, the expression of *NEUROD 1* and *TBX19* based on RT-PCR analysis was not altered (Table 4.4).

Although the expression of *NR3C1* was not affected based on microarray analysis (Table 4.8), the expression was evaluated by RT-PCR because glucocorticoid receptor complex represses the *POMC* gene through a negative glucocorticoid response element of *POMC* promoter (Drouin et al., 1993). However, RT-PCR analysis found no difference in *NR3C1* abundance in the pituitaries of HE and LE steers (Table 4.4).

4.5 Discussion

The pituitary is an endocrine gland composed of anterior, intermediate, and posterior lobes, with the anterior lobe occupying approximately 80% of the entire gland.

The anterior lobe is composed of five tropic cell types, which together secrete six hormones: corticotrophs (ACTH), gonadotrophs (FSH and LH), lactotrophs (prolactin), somatotrophs (GH), and thyrotrophs (TSH). Previous studies show that hormone production by all five anterior pituitary cell types is affected by the consumption of ergot alkaloids in cattle (Browning et al., 1997; Browning et al., 1998), with decreased concentrations of serum prolactin one of the most common serological signs (Hurley et al., 1980; Lipham et al., 1989).

To our knowledge, the effect of ergot alkaloid consumption on pituitary transcriptomic profiles has not been reported. To obtain this information, we conducted transcriptome analysis of pituitaries collected from previously described (Brown et al., 2009) beef steers suffering from fescue toxicosis induced by summer-long grazing (89 to 105 d) of HE and LE pastures. Importantly, concentrations of prolactin in the serum of HE steers were only approximately 10% of those of the LE steers (Brown et al., 2009), and the glucocorticoid receptor-mediated pathway was implicated in observed changes in carbohydrate metabolism in HE steers (Liao et al., 2015). As noted in the Introduction, the goal of the current research was to determine whether gene expression profiles differed between whole pituitaries of HE and LE steers using transcriptome and targeted gene expression analysis and to identify specific candidate molecules and signaling pathways responsible for the altered physiology of steers consuming ergot alkaloid-containing tall fescue. The global hypothesis tested was that consumption of endophyte-infected tall fescue would alter pituitary transcriptome profiles. At the $P < 0.001$ level, the microarray analysis approach revealed the differential expression of 542 RNA transcripts by the pituitary. Importantly, the pattern of altered gene expression was clearly

dependent on treatment according to hierarchical cluster analysis (Figure 4.4). Thus, the first salient finding of this study is that summer-long grazing of endophyte-infected tall fescue alters the pituitary transcriptome; thus, the global hypothesis is accepted.

More specifically, given that the serum prolactin concentrations of HE steers were only approximately 10% of those of the LE steers (Brown et al., 2009), and that the glucocorticoid receptor-mediated pathway was implicated in observed changes in carbohydrate metabolism in HE steers (Liao et al., 2015), the expectation was that the expression pattern for pituitary genes responsible for the production and secretion of prolactin would be consistent with a down-regulated capacity, whereas that for POMC/ACTH would be consistent with an up-regulated capacity. Conclusions reached about these hypotheses, as well as the possible roles of miRNA in these processes, are discussed below.

4.5.1 Fescue toxicosis and prolactin synthesis and secretion

The effect of ergot alkaloid consumption on prolactin production and secretion is best understood through the interactive pathway of dopamine receptors located on the surface of lactotrophs. Dopamine is one of the most influential regulators of prolactin secretion. Activation of the dopamine receptor suppresses *PRL* gene expression via the inhibition of adenylyl cyclase and prolactin exocytosis through modification of several potassium and calcium channels (Fitzgerald and Dinan, 2008). One way by which ergot alkaloid consumption directly affects lactotrophs is through the binding and stimulation of dopamine type two receptors (*DRD2*) on the cell surface (Fitzgerald and Dinan, 2008). Ergot alkaloids ingested with consumption of endophyte-infested tall fescue structurally resemble various biogenic amines, such as dopamine (Strickland et al., 2011). These

ergot amines can bind to dopamine type two receptors, stimulate the receptors, and reduce basal level prolactin production and secretion as described above (Larson et al., 1999). Consistent with this understanding, the HE steers in this study had serum prolactin concentrations that were only 10% of those of the LE steers (Brown et al., 2009). The lower prolactin found in serum of steers exposed to HE pasture directly corresponded to the microarray and real-time RT-PCR results regarding the gene expression of *DRD2*, *POU1F1* (a.k.a. Pit1), *PRL* and *PRLR* genes (Table 4.4). Based on real-time RT-PCR results, the expression of these genes decreased by approximately 53%, 32%, 82%, and 22% (long isoform of prolactin receptor with tendency to differ), respectively, in HE vs. LE steers. *POU1F1* plays a pivotal role in *PRL* expression by binding to specific sites of promoter elements in the *PRL* gene (Fox et al., 1990). Therefore, decreased expression of *POU1F1* might explain reduced *PRL* mRNA expression in HE steers to a certain extent.

An apparently associated finding was the accompanying down regulation of both *DRD2* and *PRLR* genes. Although speculative, a decrease in the expression of *DRD2* may have been a preventive measure by the lactotrophic cells to counteract the suppression of prolactin production due to the activation of the dopamine receptors, whereas the down regulation of prolactin receptor mRNA in pituitary tissue may be the result of a decreased requirement for prolactin binding in a prolactin-poor environment. Additionally, expression of *GAL* (galanin/GMAP prepropeptide) and *VIP* (vasoactive intestinal peptide) also decreased in HE vs. LE steers according to both microarray and real-time RT-PCR results (Table 4.4). Galanin is known to stimulate prolactin release (Koshiyama et al., 1987; Wynick et al., 1998), although the mechanism has not been clearly defined. Additionally, galanin may directly stimulate prolactin expression and act as a lactotroph

growth factor, particularly when exposure to estrogen is high (Wynick et al., 1998). Vasoactive intestinal peptide also stimulates prolactin secretion in multiple species, with receptors found on lactotrophs (Gourdji et al., 1979; Frawley and Neill, 1981; Samson et al., 1981; Macnamee et al., 1986). Although the mechanism by which vasoactive intestinal peptide stimulates prolactin release is not well delineated, as for galanin, cAMP accumulation and a delayed increase in calcium concentration were observed in the process (Samson et al., 1981; Bjøro et al., 1987). Thus, our hypothesis that at least the pituitary genes responsible for the production and secretion of prolactin would be down-regulated is also accepted.

In addition to prolactin, *POU1F1* activates growth hormone (*GH1*) promoter transcriptionally (Mangalam et al., 1989) and is involved in thyrotropin-releasing hormone (TRH) stimulation of the beta subunit of thyroid-stimulating hormone (*TSHB*) expression (Steinfelder et al., 1991). However, RT-PCR (Table 4.4) analysis corroborated the microarray (Table 4.8) findings that neither *GH1* nor *TSHB* was differentially expressed in HE vs. LE steers.

Although best known for the role in regulating lactation, prolactin affects a wide variety of biological functions (Lamberts and Macleod, 1990; Freeman et al., 2000), including reproduction, osmoregulation, antiangiogenic activity, regulation of immune responses, regulation of insulin release, and control of growth. With regard to growth, prolactin is associated with food intake and body weight and may interact with hypothalamic neurons responsible for appetite regulation (Ben-Jonathan et al., 2006; Naef and Woodside, 2007). Moreover, as described in detail previously (Brown et al., 2009), the average daily gain of HE steers was 31% less than that of LE steers ($P < 0.05$),

and the final body weight of HE steers was 7.4% less than that of LE steers ($P < 0.05$). Hence, reduced prolactin concentrations in HE steers might account for these observations to a certain degree.

4.5.2 Fescue toxicosis, POMC/ACTH synthesis, and gluconeogenesis

As noted in the Introduction, increased mitochondrial mass and capacity for ATP synthesis and amino acid-derived gluconeogenesis (Brown et al., 2009) are postulated to be coordinated through the glucocorticoid receptor-mediated pathway (Liao et al., 2015). Therefore, a reasonable hypothesis is that the capacity for glucocorticoid synthesis (POMC/ACTH production) would be elevated in the pituitaries of HE vs. LE steers. However, although we did not measure ACTH stimulation of cortisol release by the adrenal glands, canonical pathway analysis of pituitary DEGs indicated (z-score less than -2.00) the down-regulation of the corticotropin-releasing hormone (CRH) signaling pathway (*BDNF*, *POMC*, *ADCY8*, *PRKCA*, and *PRKAR1A*) in HE steers (Table 4.3). As part of the hypothalamic-pituitary-adrenal axis, the primary function of CRH is to stimulate ACTH production from the pituitary through interaction with CRHR1, the predominant pituitary-expressed CRH receptor. According to the microarray and RT-PCR analyses (Table 4.4, Table 4.8), *CRHR1* mRNA expression level was not affected in HE vs. LE steers, whereas *CRHR2* was not qualified for RT-PCR analysis because of the low expression level. These findings are consistent with the understanding that *CRHR1* is highly expressed by the pituitary, whereas *CRHR2* is predominately expressed by brain and peripheral tissues (Holsboer, 1999). ACTH is synthesized within the anterior pituitary as part of the much larger precursor molecule proopiomelanocortin (POMC), which is cleaved into smaller peptide hormones in a tissue-specific manner by proprotein

convertases. In pituitary corticotrophs, proprotein convertase 1 (encoded by the *PCSK1* gene) alone is expressed and cleaves POMC, producing ACTH, β-endorphin, β-lipotrophin, amino-terminal peptide, and joining peptide (Millington, 2007). According to the microarray and real-time RT-PCR analyses (Table 4.4), the abundance of both *POMC* and *PCSK1* mRNA was reduced in the pituitaries of HE vs. LE steers. Thus, the hypothesis that expression of pituitary genes responsible for the production of POMC/ACTH would be increased is rejected.

Despite the importance to adrenal steroidogenesis, research describing the effects of fescue toxicosis on blood ACTH is lacking. Moreover, although studies have been conducted to better understand the relationship between fescue toxicosis and circulating cortisol in cattle, their results are discordant (Aldrich et al., 1993; Schuenemann et al., 2005; Looper et al., 2010). To resolve the apparent enigma that HE steers displayed a reduced potential for pituitary synthesis of ACTH (this study), yet increased hepatic gluconeogenesis capacity (Brown et al., 2009; Liao et al., 2015), further research is required.

4.5.3 Role of miRNAs in regulating prolactin and POMC/ACTH pathways

Messenger RNA abundance is regulated by a combination of pre-transcription and post-transcription events. Transcription factors contribute to mRNA abundance at the pre-transcription level by binding to DNA and either positively or negatively regulating gene transcription (Latchman, 1997). MicroRNAs regulate mRNA abundance at the post-transcriptional level through complementary binding of target mRNA transcripts, resulting in repressed translation or enhanced degradation of bound mRNA (Engels and Hutvagner, 2006). Thus, decreased expression of a given miRNA would result in

increased target mRNA abundance and vice versa. miR-544A, which putatively regulates multiple transcription factors and stimulators (*ESR1* (Waterman et al., 1988), *EGF* (Murdoch et al., 1982), *IKZF1* (Ezzat et al., 2005), *POU1F1* (Nelson et al., 1988), *PREB* (Fliss et al., 1999), *VIP* (Bredow et al., 1994)) of the prolactin gene (Table 4.5), was down-regulated 38% in HE vs. LE steers; however, expression of *PRL* decreased in HE vs. LE steers. Inconsistency between the abundance of miR-380 and its target gene was also found. Because miR-380 is predicted to target *PCSK1* and two *POMC* transcription factors, *NEUROD1* (Poulin et al., 1997) and *TBX19* (Lamonerie et al., 1996) (Table 4.5), we expected the decrease in expression of miR-380 (42%) in the pituitaries of HE vs. LE steers to result in increased expression of *POMC* and *PCSK1*. However, microarray (Table 4.8) and RT-PCR (Table 4.4) results showed no difference in expression level of *NEUROD1* and *TBX19* mRNA in pituitaries of HE vs. LE steers, whereas both *POMC* and *PCSK1*were down-regulated.

Although evidence shows that miRNAs can also up-regulate gene expression (Vasudevan et al., 2007), an alternative explanation to the above inconsistencies could be due to the stringency level of P-values that was applied to the microarray analysis. Any given gene is usually regulated by numerous miRNAs, and the complements of these miRNAs decide the fate of the transcription of the gene. Thus, a stringent significant cutoff criterion ($P < 0.001$) could filter out potential miRNAs targeting genes of interest. For example, one striking finding listed in Table 4.5 is that no DEMs were identified that targeted *DRD2, PRL,* or *POMC*. However, when the P-value was relaxed to 0.05, then multiple miRNAs predicted to target *DRD2* (miR-141, miR-214, miR-584, miR-631, miR-2316, miR-2350, miR-2373, miR-2382, miR-2418, miR-2464) were identified (data

not shown). Additionally, miR-2335 and miR-2399 (predicted to target both *VIP* and its transcription factor NURR1 (Luo et al., 2007) (encoded by *NR4A2*)) also became candidate regulators of *PRL* expression. Collectively, the evidence suggested that altered expression of miRNAs might have affected mRNA abundance by affecting both pre- and post-transcription events of genes regulating prolactin and POMC/ACTH pathways.

This experiment is part of a comprehensive study to understand the whole body and tissue-specific effects of ergot alkaloid consumption in cattle (Brown et al., 2009; Jackson et al., 2015; Liao et al., 2015). The unique pituitary-specific findings of this study are an important contribution to our understanding of how ergot alkaloids exert their deleterious effects on cattle production. In summary, the findings indicate that anterior pituitary functions were globally impaired in steers consuming high-toxic endophyte-infected tall fescue. In addition to inhibiting the abilities to synthesize and secrete prolactin (a function of lactotrophs), ACTH synthesis capacity (a function of corticotrophs) might have been reduced. Canonical pathway analysis also indicated that growth hormone signaling and GnRH signaling were altered in HE vs. LE steers (Table 4.3). A larger implication of this research may be that it allows for selective breeding for genotypes with a higher resistance to endophyte toxicosis, because the specific genes and networks of genes have now been identified that are susceptible to ergot alkaloids contained in endophyte-infected tall fescue. Likewise, with the identification of putative ergot alkaloid sensitive mechanisms within the pituitary gland, this new knowledge may help to develop dietary treatments that ameliorate the effects of ergot alkaloid ingestion (Matthews and Bridges, 2014).

Table 4.1. Top seven IPA-identified canonical pathways of genes differentially expressed by pituitary tissue of steers grazing high (HE) vs. low (LE) endophyte-infected forages.

Canonical Pathway	Number [1]	Gene Symbol	Ratio[2]	-log (P-value)
Axonal Guidance Signaling	26	*ITSN1,BDNF,PIK3R1,UNC5B,GNB5, ABLIM1,SEMA4C,PLCD1,GNB4,SRGAP2,ABLIM2,ACE,GNG12,PRKCA,EPHA7,PLXNC1,PRKCQ,FES,PAK6,FGFR1,ITGA2,GNG3,PLCL2,WIPF1,SEMA3C,PRKAR1A*	0.06	5.38
Role of NFAT in Cardiac Hypertrophy	16	*MAP2K6,PRKCQ,FGFR1,PIK3R1,SLC8A3,GNB5,GNG3,PLCL2,PLCD1,GNB4,MAPK10,RCAN3,ADCY8,GNG12,PRKAR1A,PRKCA*	0.08	5.33
P2Y Purigenic Receptor Signaling Pathway	13	*PRKCQ,FGFR1,PIK3R1,CREB3,GNB5,GNG3,PLCL2,PLCD1,GNB4,ADCY8,GNG12,PRKCA,PRKAR1A*	0.09	5.04
Cardiac Hypertrophy Signaling	17	*MAP2K6,DIRAS3,PIK3R1,FGFR1,IL6R,GNB5,GNG3,MAP3K5,PLCL2,PLCD1,GNB4,RHOQ,MAPK10,ADCY8,MAP3K3,GNG12,PRKAR1A*	0.07	4.80
Tec Kinase Signaling	14	*GNB4,PRKCQ,RHOQ,PAK6,DIRAS3, PIK3R1,FGFR1,ITGA2,GNB5,MAPK10,GNG3,FRK,GNG12,PRKCA*	0.08	4.77
ErbB Signaling	10	*GNB4,PRKCQ,RHOQ,PAK6,DIRAS3, PIK3R1,FGFR1,GNB5,MAPK10,GNG3,ADCY8,GNG12,PRKCA*	0.10	4.41
CXCR4 Signaling	13	*GNB4,PRKCQ,RHOQ,PAK6,DIRAS3, PIK3R1,FGFR1,GNB5,MAPK10,GNG3,ADCY8,GNG12,PRKCA*	0.08	4.14

[1]The number of genes (listed in the "Symbol" column) associated with the particular canonical pathway.

Table 4.1 (continued)

[2]The ratio is calculated as the number of genes in a given pathway that meet cutoff criteria (e.g., the ANOVA P-value for the differential expression between HE and LE groups is less than 0.001) divided by the total number of genes that make up that pathway.

Table 4.2. IPA-identified canonical pathways of genes central to prolactin production, secretion, or signaling differentially-expressed by pituitary tissue of steers grazing high (HE) vs. low (LE) endophyte-infected forages.

Canonical Pathway	Number [1]	Gene Symbol	Ratio[2]	-log (P-value)
Dopamine Receptor Signaling	4	*PRL,ADCY8,DRD2,PRKAR1A*	0.04	0.91
Gαi Signaling	9	*GABBR2,GNB4,GNB5,HTR1F,GNG3, ADCY8,DRD2,GNG12,PRKAR1A*	0.07	2.95
cAMP-mediated Signaling	9	*PDE8A,GABBR2,PKIB,CREB3,HTR1F ,ADCY8,DRD2,CNGA3,PRKAR1A*	0.04	1.31
Protein Kinase A Signaling	20	*PRKCQ,PTPRD,CREB3,MYLK3,GNB5 ,GNG3,PLCL2,CNGA3,PDE8A,PLCD1 ,GNB4,DUSP10,ADCY8,PTPRN,EYA1, KDELR2,GNG12,TCF7L2,PRKCA,PR KAR1A*	0.05	3.45
Prolactin Signaling	6	*PRKCQ,PRL,PIK3R1,FGFR1,PRLR,P RKCA*	0.07	2.04

[1]The number of genes (listed in the "Symbol" column) associated with the particular canonical pathway.

[2]The ratio is calculated as the number of genes in a given pathway that meet cutoff criteria (e.g., the ANOVA P-value for the differential expression between HE and LE groups is < 0.001) divided by the total number of genes that make up that pathway.

Table 4.3. IPA-identified canonical pathways of genes involved in signaling of selected pituitary-derived hormones differentially-expressed by pituitary tissue of steers grazing high (HE) vs. low (LE) endophyte-infected forages.

Canonical Pathway	Number [1]	Gene Symbol	Ratio[2]	-log (P-value)
Melanocyte Development and Pigmentation Signaling	7	*PIK3R1,FGFR1,CREB3,POMC,RPS6KA5,ADCY8,PRKAR1A*	0.07	2.38
Corticotropin-releasing Hormone Signaling	7	*PRKCQ,BDNF,CREB3,POMC,ADCY8,PRKCA,PRKAR1A*	0.06	1.87
Growth Hormone Signaling	6	*IGF2,PRKCQ,PIK3R1,FGFR1,RPS6KA5,PRKCA*	0.07	2.06
GnRH Signaling	10	*MAP2K6,PRKCQ,PAK6,CREB3,MAPK10,MAP3K5,ADCY8,MAP3K3,PRKCA,PRKAR1A*	0.07	3.26

[1]The number of genes (listed in the "Symbol" column) associated with the particular canonical pathway.

[2]The ratio is calculated as the number of genes in a given pathway that meet cutoff criteria (e.g., the ANOVA P-value for the differential expression between HE and LE groups is less than 0.001) divided by the total number of genes that make up that pathway.

Table 4.4. Comparison of microarray and real-time RT-PCR identification of selected genes by pituitary tissue of steers grazing high (HE) vs. low (LE) endophyte-infected forages.

Gene	Gene Name	Microarray			Real-time RT-PCR		
		Change[2]	Ratio[3]	P-value	Change[2]	Ratio[3]	P-value
ACTB[1]	Actin, beta	1.03	1.03	0.084	1.01	1.01	0.568
PPIA[1]	Peptidylprolyl isomerase A	-1.07	0.93	0.441	1.00	1.00	0.422
UBC[1]	Ubiquitin C	1.00	1.00	0.994	1.00	1.00	0.816
DRD2	Dopamine receptor D2	-1.76	0.57	0.001	-2.14	0.47	0.001
PRL	Prolactin	-1.23	0.81	0.001	-5.67	0.18	0.001
PRLR	Prolactin receptor	-1.31	0.76	0.001	NA	NA	NA
s-PRLR	Prolactin receptor short isoform	NA	NA	NA	-1.20	0.83	0.210
l- PRLR	Prolactin receptor long isoform	NA	NA	NA	-1.29	0.78	0.062
POU1F1	POU class 1 homeobox 1	-1.30	0.77	0.003	-1.47	0.68	0.038
GAL	Galanin/GMAP prepropeptide	-1.34	0.74	0.009	-2.35	0.43	0.019
VIP	Vasoactive intestinal peptide	-1.76	0.57	0.003	-2.08	0.48	0.045
POMC	Proopiomelanocortin	-1.25	0.80	0.001	-2.27	0.44	0.006
PCSK1	Proprotein convertase subtilisin/kexin type 1	-1.72	0.58	0.001	-2.02	0.5	0.001
GH1	Growth Hormone 1	1.01	1.01	0.728	1.20	1.20	0.436
TSHB	Thyroid stimulating hormone beta	1.00	1.00	0.999	1.14	1.14	0.418

Table 4.4 (continued)

TBX19	T-Box 19	-1.14	0.88	0.104	-1.20	0.84	0.217
NeuroD 1	Neuronal differentiation 1	1.19	1.19	0.178	1.18	1.18	0.415
NR3C1	Nuclear receptor subfamily 3 group C member 1	1.14	1.14	0.112	1.33	1.33	0.270
CRHR1	Corticotropin-releasing hormone receptor 1	1.19	1.19	0.106	1.39	1.39	0.192

[1]Expression reference genes.

[2]Data are expressed as fold change in HE relative to LE expression.

[3]Data are expressed as ratio of HE relative to LE expression.

Table 4.5. Predicted relationship between differentially-expressed mRNA of prolactin and ACTH pathway genes, including transcription factors (TF), transcription stimulators (TS), and transcription inhibitors (TI), known to be targets of microarray-identified differentially-expressed miRNAs (DEMs)[1].

Gene Symbol	Gene Description	DEM ($P < 0.001$)[2,3]
PRL	Prolactin	
PRLR	Prolactin Receptor	miR-329B, miR-380, miR-544A, miR-2318, miR-2356
DRD2	Dopamine Receptor D2	
POU1F1 (TF for *PRL*)	POU Class 1 Homeobox 1	miR-544A
VIP	Vasoactive Intestinal Peptide	miR-544A, miR-2400
ESR1 (TF for *PRL*)	Estrogen Receptor 1	miR-329B, miR-380, miR-544A, miR-2356
PREB (TF for *PRL*)	Prolactin Regulatory Element Binding	miR-544A, miR-2400
EGF (TS for *PRL*)	Epidermal Growth Factor	miR-380, miR-544A, miR-2356
IKZF1 (TS for *PRL*)	IKAROS Family Zinc Finger 1	miR-380, miR-544A, miR-2400
PKIA (TI for *PRL*)	CAMP-Dependent Protein Kinase Inhibitor Alpha	miR-329B, miR-380, miR-544A, miR-2318, miR-2356, miR-2400
POMC	Proopiomelanocortin	
PCSK1	Proprotein Convertase Subtilisin/Kexin Type 1	miR-380
TBX19 (TF for *POMC*)	T-Box 19	miR-380
NEUROD1 (TF for *POMC*)	Neuronal Differentiation 1	miR-380, miR-544A, miR-2318
JUN (TF for *POMC*)	Jun Proto-Oncogene, AP-1 Transcription Factor Subunit	miR-2400
LEP (TS for *POMC*)	Leptin	miR-544A
LIF (TS for *POMC*)	Leukemia Inhibitory Factor	miR-2400
NR3C1 (TI for *POMC*)	Nuclear Receptor Subfamily 3 Group C Member 1	miR-380, miR-544A, miR-2318, miR-2356
SMARCA4 (TI for *POMC*)	SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A, Member 4	miR-329B
HDAC2 (TI for *POMC*)	Histone Deacetylase 2	miR-380, miR-2356

Table 4.5 (continued)

[1]Putative gene targets of DEMs were identified using TargetScan (Release 7.1, http://www.targetscan.org).

[2]miR-329B: GenBank accession number is NR_031209 and is known as miR-329 by TargetScan.

[3]miR-544A: GenBank accession number is NR_031187 and is known as miR-544 by TargetScan.

Table 4.6. Primer sets used for quantitative real-time RT-PCR analysis of the selected differentially expressed genes and reference genes.

Gene	Gene Name	Primer & Accession number[1]	Sequence (5' to 3' direction)	Amplicon length (bp)	Product identity (%)[2]
Actb	Actin, beta	*NM_173979.3*			
		Forward	GAGCGGGAAATCGTCCGTGAC	278	99
		Reverse	GTGTTGGCGTAGAGGTCCTTGC		
Ppia	Peptidylprolyl isomerase A	*NM_178320.2*			
		Forward	GGCAAGTCCATCTATGGCGA	239	99
		Reverse	TTGCTGGTCTTGCCATTCCT		
Ubc	Ubiquitin C	*NM_001206307.1*			
		Forward	TAGGGGTGGGTTAGAGTTCAAG	258	100
		Reverse	ACCACCTCCCTGCTGGTATT		
Drd2	Dopamine receptor D2	*NM_174043.2*			
		Forward	CGACCTTTCTCTGGGGCTTT	234	100
		Reverse	TTGGGCTTCTGCTTCTCTGG		
Prl	Prolactin	*NM_173953.2*			
		Forward	AGAACAAGCCCAACAGACCC	252	99
		Reverse	AGTCCTGACCACACAGGGTA		

Table 4.6 (continued)

s-Prlr	Short Prolactin receptor	*NM_174155.3*			
		Forward	GCCATCCTTTCTGCTGTCAT	151	99
		Reverse	AAGGCGAGAAGGCTGTGATA		
l-Prlr	Long Prolactin receptor	*NM_001039726.2*			
		Forward	GCCATCCTTTCTGCTGTCAT	136	100
		Reverse	CCCTTCTCCAGCAGATGAAC		
Pou1f1	POU class 1 homeobox 1	*AH012495.1*			
		Forward	AAGCAAGAGGTTTGAAGTTTGGT	401	99
		Reverse	TGCTCTTTAGCCAGCCTTGA		
Gal	Galanin/GMAP prepropeptide	*NM_173914.2*			
		Forward	CACCGGTGAAGGAGAAGAGAG	230	100
		Reverse	GGCGTCTTTGAGATGCAGGAA		
Vip	Vasoactive intestinal peptide	*NM_173970.3*			
		Forward	CTGGTTCAGCTGTAAGGGCA	325	100
		Reverse	TCAGCCAGCGCATCTTGTAA		
Pomc	Proopiomelanocortin	*NM_174151.1*			
		Forward	AGCTTCCCCGTGACAGAGC	317	99

Table 4.6 (continued)

		Reverse	CTGCTACCATTCCGACGGC		
Pcsk1	Proprotein convertase sutilisin/kexin type 1	*NM_174412.2*			
		Forward	TGATCGTGTGATATGGGCGG	277	100
		Reverse	GGCCTCCGGATCATAGTTGG		

[1]The contents in the parentheses associated with each gene symbol are the accession numbers of the sequences retrieved from the NCBI RefSeq database and used as templates for designing primers and probes.

[2]All the real-time RT-PCR products were validated by sequencing. The identity values (%) presented are the base-pair ratios between the number of identical base pairs and the total amplicon length.

Table 4.7. List of differentially expressed pituitary genes (P < 0.001, 542 genes) collected from steers grazing high- (HE, n = 8) or low- (LE, n = 8) endophyte-infected forages.

Transcript ID	Gene Symbol	Gene assignment	p-value	False discovery Rate	Ratio(HE vs.LE)	Fold-Change(HE vs. LE)
12876894	GPX3	glutathione peroxidase 3 (plasma)	8.90E-10	2.37E-05	0.479140613	-2.08707
12851243	ASB4	ankyrin repeat and SOCS box containing 4	1.73E-08	0.000230196	0.526931467	-1.89778
12898500	EPHA7	EPH receptor A7	2.67E-08	0.000237549	1.797668783	1.79767
12835339	COPA	coatomer protein complex, subunit alpha	5.72E-08	0.000332512	0.906996572	-1.10254
12798282	CHL1	cell adhesion molecule with homology to L1CAM (close homolog of	6.23E-08	0.000332512	0.532076215	-1.87943
12871398	PLA2G12A	phospholipase A2, group XIIA	9.95E-08	0.000387737	0.761666832	-1.31291
12776100	CPS1	carbamoyl-phosphate synthase 1, mitochondrial	1.02E-07	0.000387737	0.425693135	-2.34911
12721755	MMP16	matrix metallopeptidase 16 (membrane-inserted)	2.29E-07	0.000764464	1.30842271	1.30842
12902267	BHLHB9	basic helix-loop-helix domain containing, class B, 9	4.95E-07	0.00146659	0.712230421	-1.40404

Table 4.7 (continued)

12730735	DRD2	dopamine receptor D2	5.55E-07	0.00148011	0.569216758	-1.7568
12737221	FAM163A	family with sequence similarity 163, member A	8.61E-07	0.00192236	0.532013939	-1.87965
12780430	PTPRN	protein tyrosine phosphatase, receptor type, N	9.71E-07	0.00192236	0.776578396	-1.2877
12866683	SLC41A2	solute carrier family 41, member 2	1.12E-06	0.00192236	0.753801042	-1.32661
12722069	CDH17	cadherin 17, LI cadherin (liver-intestine)	1.15E-06	0.00192236	0.309513198	-3.23088
12826450	UNC5B	unc-5 homolog B (C. elegans)	1.15E-06	0.00192236	0.617078258	-1.62054
12767970	NSF	N-ethylmaleimide-sensitive factor	1.20E-06	0.00192236	0.856927401	-1.16696
12836423	DIRAS3	DIRAS family, GTP-binding RAS-like 3	1.26E-06	0.00192236	0.731042247	-1.36791
12729629	REXO2	REX2, RNA exonuclease 2 homolog (S. cerevisiae)	1.30E-06	0.00192236	0.638740914	-1.56558
12890848	RORB	RAR-related orphan receptor B	1.57E-06	0.00209906	1.373516945	1.37352
12679305	LNP1	leukemia NUP98 fusion partner 1	1.62E-06	0.00209906	0.838609585	-1.19245
12866960	PLEKHA5	pleckstrin homology domain containing A5	1.65E-06	0.00209906	1.257116853	1.25712

Table 4.7 (continued)

12765517	RPRML	reprimo-like	1.81E-06	0.00219319	0.772260406	-1.2949
12821658	ABLIM1	actin binding LIM protein 1	2.06E-06	0.00230454	1.308681268	1.30868
12909850	SLITRK2	SLIT and NTRK-like family, member 2	2.07E-06	0.00230454	1.349001537	1.349
12876760	PAM	peptidylglycine alpha-amidating monooxygenase	2.21E-06	0.0023559	0.5161343 6	-1.93748
12706637	FAM98A	family with sequence similarity 98, member A	2.85E-06	0.00292358	0.814730324	-1.2274
12731709	ZW10	ZW10, kinetochore associated, homolog (Drosophila)	3.33E-06	0.00326731	0.826111 74	-1.21049
12722190	MRPL15	mitochondrial ribosomal protein L15	3.46E-06	0.00326731	0.746246381	-1.34004
12883237	PCSK1	proprotein convertase subtilisin	3.55E-06	0.00326731	0.580548154	-1.72251
12901396	PKIB	protein kinase (cAMP-dependent, catalytic) inhibitor beta	3.92E-06	0.00348198	0.710292854	-1.40787
12844085	PEA15	phosphoprotein enriched in astrocytes 15	4.07E-06	0.00350175	1.182574062	1.18257
12725728	EFR3A	EFR3 homolog A (S. cerevisiae)	4.33E-06	0.00350945	0.759999696	-1.31579
12796976	SLC6A11	solute carrier family 6 (neurotransmitter transporter, GABA)	4.34E-06	0.00350945	1.353280691	1.35328

Table 4.7 (continued)

12681884	PLSCR4	phospholipid scramblase 4	4.85E-06	0.00380365	1.340186152	1.34019
12698445	ODC1	ornithine decarboxylase 1	5.00E-06	0.00381304	0.720964939	-1.38703
12738110	APOBEC4	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-	5.77E-06	0.00411905	0.58433742	-1.71134
12690836	RORA	RAR-related orphan receptor A	5.81E-06	0.00411905	1.179369061	1.17937
12804415	PRL	prolactin	6.12E-06	0.00411905	0.814617496	-1.22757
12884669	DPYSL3	dihydropyrimidinase-like 3	6.12E-06	0.00411905	0.623526918	-1.60378
12820770	TCF7L2	transcription factor 7-like 2 (T-cell specific, HMG-box	6.28E-06	0.00411905	1.215108169	1.21511
12681044	ADPRH	ADP-ribosylarginine hydrolase	6.55E-06	0.00411905	0.79231141	-1.26213
12816646	WBSCR22	Williams Beuren syndrome chromosome region 22	6.57E-06	0.00411905	0.832625602	-1.20102
12909727	GPR101	G protein-coupled receptor 101	6.64E-06	0.00411905	0.372790749	-2.68247
12684305	ITSN1	intersectin 1 (SH3 domain protein)	7.11E-06	0.00423958	1.169628947	1.16963
12694513	HEATR4	HEAT repeat containing 4	7.15E-06	0.00423958	1.248296076	1.2483

Table 4.7 (continued)

12854099	ASNS	asparagine synthetase (glutamine-hydrolyzing)	8.47E-06	0.00486962	0.823092688	-1.21493
12835795	CD247	CD247 molecule	8.58E-06	0.00486962	1.181139563	1.18114
12760666	CLTC	clathrin, heavy chain (Hc)	8.86E-06	0.00492327	0.913567389	-1.09461
12891421	NFIB	nuclear factor I	9.41E-06	0.00512338	1.240334692	1.24033
12796050	EXOG	endo	9.64E-06	0.00514056	0.828665186	-1.20676
12744882	CIT	citron (rho-interacting, serine	9.90E-06	0.00518005	0.789914373	-1.26596
12728901	SORL1	sortilin-related receptor, L(DLR class) A repeats contai	1.03E-05	0.00529555	1.242348685	1.24235
12863173	IQSEC3	IQ motif and Sec7 domain 3	1.09E-05	0.00549229	0.695574753	-1.43766
12754444	CPNE2	copine II	1.13E-05	0.00557045	0.668847108	-1.49511
12772434	ENO3	enolase 3 (beta, muscle)	1.19E-05	0.0057655	0.652213613	-1.53324
12849880	RINT1	RAD50 interactor 1	1.22E-05	0.00577337	0.84404568	-1.18477
12845993	CADM3	cell adhesion molecule 3	1.24E-05	0.00577337	1.336918056	1.33692

Table 4.7 (continued)

12862258	RNF41	ring finger protein 41	1.26E-05	0.00577337	0.848874816	-1.17803
12760918	TIMP2	TIMP metallopeptidase inhibitor 2	1.36E-05	0.00592615	0.828919338	-1.20639
12890327	LPPR1	lipid phosphate phosphatase-related protein type 1	1.36E-05	0.00592615	0.703650539	-1.42116
12721605	FABP5	fatty acid binding protein 5 (psoriasis-associated)	1.38E-05	0.00592615	0.630250777	-1.58667
12772567	CDR2L	cerebellar degeneration-related protein 2-like	1.39E-05	0.00592615	0.610884744	-1.63697
12818366	VGF	VGF nerve growth factor inducible	1.40E-05	0.00592615	0.688477638	-1.45248
12909004	KLHL13	kelch-like 13 (Drosophila)	1.42E-05	0.00592615	0.647261759	-1.54497
12681831	BCL6	B-cell CLL	1.45E-05	0.00594922	1.390644578	1.39064
12887101	CLINT1	clathrin interactor 1	1.51E-05	0.00611067	0.902535221	-1.10799
12822767	GFRA1	GDNF family receptor alpha 1	1.57E-05	0.00625298	1.370279729	1.37028
12825420	ACSL1	acyl-CoA synthetase long-chain family member 1	1.60E-05	0.00628789	1.263664957	1.26366
12744620	KSR2	kinase suppressor of ras 2	1.64E-05	0.00632783	0.776831769	-1.28728

Table 4.7 (continued)

12734598	PRELP	proline	1.68E-05	0.00640273	1.297572242	1.29757
12812405	TPST1	tyrosylprotein sulfotransferase 1	1.70E-05	0.00640273	0.755635149	-1.32339
12780755	RCAN3	RCAN family member 3	1.78E-05	0.00654189	0.616663481	-1.62163
12830994	LRRC10B	leucine rich repeat containing 10B	1.79E-05	0.00654189	0.774521346	-1.29112
12717875	LOC100335867	uncharacterized LOC100335867	1.87E-05	0.00672986	0.702864171	-1.42275
12852359	CLEC2L	C-type lectin domain family 2, member L	1.94E-05	0.00685045	0.511872892	-1.95361
12851732	COBL	cordon-bleu homolog (mouse)	1.95E-05	0.00685045	1.226814735	1.22681
12800654	FAM19A1	family with sequence similarity 19 (chemokine (C-C motif)-li	2.03E-05	0.00698948	0.491956511	-2.0327
12791580	SERPINA1	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, a	2.04E-05	0.00698948	0.37702557	-2.65234
12914851			2.18E-05	0.00725635	0.338304149	-2.95592
12894663	KANK1	KN motif and ankyrin repeat domains 1	2.22E-05	0.00725635	1.228496701	1.2285
12880336	RHOBTB3	Rho-related BTB domain containing 3	2.22E-05	0.00725635	0.778446377	-1.28461

Table 4.7 (continued)

12690776	C10H15orf61	chromosome 10 open reading frame, human C15orf61	2.25E-05	0.00725635	0.822206144	-1.21624
12843689	IL6R	interleukin 6 receptor	2.26E-05	0.00725635	1.272669424	1.27267
12785900	SERF1A	small EDRK-rich factor 1A (telomeric)	2.29E-05	0.00725635	0.793361154	-1.26046
12900382	IPCEF1	interaction protein for cytohesin exchange factors 1	2.32E-05	0.00729635	0.665734638	-1.5021
12749473	CLEC3A	C-type lectin domain family 3, member A	2.40E-05	0.00744792	0.495554873	-2.01794
12904860	TMEM35	transmembrane protein 35	2.56E-05	0.00786195	0.773108204	-1.29348
12786942	SDHA	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	2.60E-05	0.00788154	0.907218 74	-1.10227
12901161	FAM46A	family with sequence similarity 46, member A	2.69E-05	0.00802629	0.648193162	-1.54275
12718471	PCMTD2	protein-L-isoaspartate (D-aspartate) O-methyltransferase doma	2.71E-05	0.00802629	1.159134034	1.15913
12743540	GOLGA3	golgin A3	2.92E-05	0.00856137	0.858288059	-1.16511
12914547			3.08E-05	0.00876601	0.788867502	-1.26764
12694561	SIX1	SIX homeobox 1	3.11E-05	0.00876601	0.862529973	-1.15938

Table 4.7 (continued)

12845091	GIPC2	GIPC PDZ domain containing family, member 2	3.11E-05	0.00876601	0.749198358	-1.33476
12798897	SPCS1	signal peptidase complex subunit 1 homolog (S. cerevisiae)	3.12E-05	0.00876601	0.82606397	-1.21056
12705554	KCNIP3	Kv channel interacting protein 3, calsenilin	3.19E-05	0.00885044	0.337708915	-2.96113
12900949	CLVS2	clavesin 2	3.27E-05	0.00898575	0.460095884	-2.17346
12865096	CPT1B	carnitine palmitoyltransferase 1B (muscle)	3.42E-05	0.00924483	0.766530224	-1.30458
12730077	ART5	ADP-ribosyltransferase 5	3.43E-05	0.00924483	0.702656745	-1.42317
12891381	RFX3	regulatory factor X, 3 (influences HLA class II expression)	3.65E-05	0.00967033	1.154782416	1.15478
12718040	BLCAP	bladder cancer associated protein	3.66E-05	0.00967033	1.149770218	1.14977
12885917	ZNF300	zinc finger protein 300	3.75E-05	0.00979751	0.591800019	-1.68976
12724574	DENND3	DENN	3.80E-05	0.00985362	0.761162447	-1.31378
12807840	LOC510913	nose resistant to fluoxetine protein 6-like	3.97E-05	0.0101555	0.378382264	-2.64283
12722987	AZIN1	antizyme inhibitor 1	4.01E-05	0.0101555	0.893367639	-1.11936

Table 4.7 (continued)

12824358	TNKS	tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymera	4.04E-05	0.0101555	0.860067085	-1.1627
12772732	PRKAR1A	protein kinase, cAMP-dependent, regulatory, type I, alpha (t	4.26E-05	0.0105979	0.91503866	-1.09285
12786113	FGF18	fibroblast growth factor 18	4.33E-05	0.0105979	0.788562687	-1.26813
12815257	STX1B	syntaxin 1B	4.33E-05	0.0105979	1.19426086	1.19426
12782974	MFSD6	major facilitator superfamily domain containing 6	4.39E-05	0.0106441	0.862009517	-1.16008
12703883	GFPT1	glutamine--fructose-6-phosphate transaminase 1	4.43E-05	0.0106441	0.882378893	-1.1333
12808014	SLC39A6	solute carrier family 39 (zinc transporter), member 6	4.48E-05	0.0106789	0.83467577	-1.19807
12709784	GPC5	glypican 5	4.72E-05	0.0110629	0.715727394	-1.39718
12727699	CREB3L1	cAMP responsive element binding protein 3-like 1	4.74E-05	0.0110629	0.674427074	-1.48274
12681063	GNB4	guanine nucleotide binding protein (G protein), beta polypeptid	4.77E-05	0.0110629	0.700461604	-1.42763
12733712	BDNF	brain-derived neurotrophic factor	4.81E-05	0.0110629	0.659565346	-1.51615
12786160	OXCT1	3-oxoacid CoA transferase 1	4.89E-05	0.0111294	0.823512942	-1.21431

Table 4.7 (continued)

12867761	COQ10A	coenzyme Q10 homolog A (S. cerevisiae)	4.94E-05	0.0111294	0.879855704	-1.13655
12706846	PREPL	prolyl endopeptidase-like	4.98E-05	0.0111294	0.857581449	-1.16607
12816678	TRAP1	TNF receptor-associated protein 1	5.01E-05	0.0111294	0.832729604	-1.20087
12771103	TRPV3	transient receptor potential cation channel, subfamily V, memb	6.00E-05	0.0132377	0.691620328	-1.44588
12719676	SLC17A9	solute carrier family 17, member 9	6.24E-05	0.0136424	0.676823532	-1.47749
12767584	MAP2K6	mitogen-activated protein kinase kinase 6	6.36E-05	0.0137836	1.493379847	1.49338
12887812	SHROOM1	shroom family member 1	6.41E-05	0.0137836	1.109131927	1.10913
12791646	WARS	tryptophanyl-tRNA synthetase	6.57E-05	0.0140166	0.859195106	-1.16388
12706360	SURF4	surfeit 4	6.81E-05	0.0143244	0.867611206	-1.15259
12707532	COX7A2L	cytochrome c oxidase subunit VIIa polypeptide 2 like	6.88E-05	0.0143244	0.798607229	-1.25218
12861735	C5H12orf23	chromosome 5 open reading frame, human C12orf23	6.89E-05	0.0143244	0.894958698	-1.11737
12677247			6.93E-05	0.0143244	1.748212016	1.74821

Table 4.7 (continued)

12844481	POU2F1	POU class 2 homeobox 1	7.08E-05	0.0144902	1.152076387	1.15208
12737098	B3GALT2	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypepti	7.16E-05	0.0144902	0.626550713	-1.59604
12758081	MYLK3	myosin light chain kinase 3	7.18E-05	0.0144902	0.704002253	-1.42045
12765456	TOM1L2	target of myb1-like 2 (chicken)	7.22E-05	0.0144902	0.841078262	-1.18895
12690792	GNB5	guanine nucleotide binding protein (G protein), beta 5	7.90E-05	0.0157305	0.901989789	-1.10866
12898520	SASH1	SAM and SH3 domain containing 1	7.98E-05	0.0157582	1.279225506	1.27923
12741946	TCN2	transcobalamin II	8.15E-05	0.0159752	0.818618663	-1.22157
12904911	HNRNPH2	heterogeneous nuclear ribonucleoprotein H2 (H')	8.20E-05	0.0159752	0.831213738	-1.20306
12713066	SLITRK6	SLIT and NTRK-like family, member 6	8.42E-05	0.016277	0.526853735	-1.89806
12848963	GNGT1	guanine nucleotide binding protein (G protein), gamma transducing	8.57E-05	0.0163351	0.471211343	-2.12219
12801704	LOC511316	MEF2B neighbor pseudogene	8.58E-05	0.0163351	0.82234137	-1.21604
12780398	TNS1	tensin 1	8.71E-05	0.0163351	1.279664216	1.27966

Table 4.7 (continued)

12831896	NRGN	neurogranin (protein kinase C substrate, RC3)	8.72E-05	0.0163351	1.663730183	1.66373
12830772	SCYL1	SCY1-like 1 (S. cerevisiae)	8.79E-05	0.0163351	0.873438728	-1.1449
12785083	PRLR	prolactin receptor	8.86E-05	0.0163351	0.762951095	-1.3107
12771233	LRRC48	leucine rich repeat containing 48	8.88E-05	0.0163351	0.798103706	-1.25297
12778650	FIGN	fidgetin	9.04E-05	0.0165243	1.271622349	1.27162
12693799	DIO2	deiodinase, iodothyronine, type II	9.17E-05	0.0166237	0.592364423	-1.68815
12708635	AFF3	AF4	9.23E-05	0.0166237	1.181992579	1.18199
12716197	TM9SF4	transmembrane 9 superfamily protein member 4	9.29E-05	0.0166237	0.880211955	-1.13609
12822603	NRAP	nebulin-related anchoring protein	9.37E-05	0.0166607	0.664721249	-1.50439
12844104	FAM19A3	family with sequence similarity 19 (chemokine (C-C motif)-li	9.43E-05	0.0166607	0.766101539	-1.30531
12696210	SLC27A2	solute carrier family 27 (fatty acid transporter), member 2	9.63E-05	0.0167793	0.653628948	-1.52992
12808788	SETBP1	SET binding protein 1	9.69E-05	0.0167793	1.142543759	1.14254

Table 4.7 (continued)

12891005	PTPRD	protein tyrosine phosphatase, receptor type, D	9.70E-05	0.0167793	1.365526998	1.36553
12855122	TES	testis derived transcript (3 LIM domains)	9.75E-05	0.0167793	0.898303105	-1.11321
12808491	ZNF521	zinc finger protein 521	9.94E-05	0.0170016	1.332127314	1.33213
12687743	NEO1	neogenin 1	0.00010109	0.0171237	1.181993977	1.18199
12886016	MFSD12	major facilitator superfamily domain containing 12	0.00010 1426	0.0171237	0.867287645	-1.15302
12892540	DMRT1	doublesex and mab-3 related transcription factor 1	0.00010 3381	0.0173439	0.851382645	-1.17456
12899957	PERP	PERP, TP53 apoptosis effector	0.00010 5136	0.0174711	0.788774166	-1.26779
12908876	MAGEE2	melanoma antigen family E, 2	0.00010 5449	0.0174711	0.861898072	-1.16023
12681492	CCDC39	coiled-coil domain containing 39	0.00010 8466	0.0178258	1.283912956	1.28391
12880093	UNC13A	unc-13 homolog A (C. elegans)	0.00010 8926	0.0178258	0.842268397	-1.18727
12705423	ANGPTL2	angiopoietin-like 2	0.00011 1958	0.0182103	0.557637402	-1.79328
12843233	AMY2B	amylase, alpha 2B (pancreatic)	0.00011 4346	0.0184009	1.275196285	1.2752

Table 4.7 (continued)

12735060	SRM	spermidine synthase	0.00011451	0.0184009	0.790713856	-1.26468
12809293	LAMA1	laminin, alpha 1	0.00011 5227	0.0184053	1.402310166	1.40231
12738701	SEC16B	SEC16 homolog B (S. cerevisiae)	0.00011 7318	0.0186278	0.694391401	-1.44011
12817661	KDELR2	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retentio	0.00011 9433	0.0188194	0.880382438	-1.13587
12891796	PALM2	paralemmin 2	0.00012 0481	0.0188194	1.200413903	1.20041
12782240	CLASP1	cytoplasmic linker associated protein 1	0.00012 1187	0.0188194	1.139714137	1.13971
12908168	MAGED1	melanoma antigen family D, 1	0.00012 1347	0.0188194	0.901233789	-1.10959
12792677	ITPK1	inositol-tetrakisphosphate 1-kinase	0.00012 2759	0.0189282	1.166775371	1.16677
12779964	4-Mar	membrane-associated ring finger (C3HC4) 4, E3 ubiquitin prote	0.00012 5636	0.0191841	0.672133351	-1.4878
12797483	RBMS3	RNA binding motif, single stranded interacting protein 3	0.00012 5856	0.0191841	1.173052176	1.17305
12895106	ACER2	alkaline ceramidase 2	0.00012 8917	0.0195389	0.770796078	-1.29736
12889108	CREB3	cAMP responsive element binding protein 3	0.00013 0329	0.0196414	0.899029947	-1.11231

Table 4.7 (continued)

12758956	CMTM4	CKLF-like MARVEL transmembrane domain containing 4	0.000135053	0.020239	1.161127315	1.16113
12692964	C10H14orf119	chromosome 10 open reading frame, human C14orf119	0.000136188	0.020295	0.865868336	-1.15491
12786958	ITGA2	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)	0.000137653	0.0203994	0.660161872	-1.51478
12875311	MAPK10	mitogen-activated protein kinase 10	0.000139985	0.0204027	0.876255236	-1.14122
12908904	ZNF711	zinc finger protein 711	0.000140058	0.0204027	1.102853191	1.10285
12767689	ATP5G1	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit	0.000140519	0.0204027	0.82350616	-1.21432
12892113	CNTNAP3	contactin associated protein-like 3	0.000140735	0.0204027	1.622823388	1.62282
12849870	TMEM213	transmembrane protein 213	0.000141848	0.0204286	1.513237041	1.51324
12863307	METTL21B	methyltransferase like 21B	0.000142445	0.0204286	0.848716317	-1.17825
12791435	MIR380	microRNA mir-380	0.000143776	0.0205093	0.584730352	-1.71019
12678498	APP	amyloid beta (A4) precursor protein	0.000149384	0.0211959	1.122431456	1.12243
12865092	RPS26	ribosomal protein S26	0.000150367	0.0212104	0.728162409	-1.37332

Table 4.7 (continued)

12689890	MYO1E	myosin IE	0.00015174	0.0212104	1.152660687	1.15266
12741977	ADRBK2	adrenergic, beta, receptor kinase 2	0.00015593	0.0212104	1.166377988	1.16638
12851480	C4H7orf25	chromosome 4 open reading frame, human C7orf25	0.00015767	0.0212104	0.800646923	-1.24899
12710335	LACC1	laccase (multicopper oxidoreductase) domain containing 1	0.00015463	0.0212104	0.829689613	-1.20527
12772085	GPR142	G protein-coupled receptor 142	0.00015854	0.0218002	0.815693952	-1.22595
12710070	COG3	component of oligomeric golgi complex 3	0.00015970	0.0218472	0.919777046	-1.08722
12697991	RHOQ	ras homolog gene family, member Q	0.00016244	0.0221085	0.790039186	-1.26576
12822932	SLC25A28	solute carrier family 25, member 28	0.00016411	0.0222217	1.133959128	1.13396
12698264	KCNIP3	Kv channel interacting protein 3, calsenilin	0.00016734	0.022473	0.548047307	-1.82466
12900780	SLC17A5	solute carrier family 17 (anion	0.00016862	0.022473	0.783293908	-1.27666
12792547	CCDC88C	coiled-coil domain containing 88C	0.00016877	0.022473	1.191649398	1.19165
12679533	TMEM45A	transmembrane protein 45A	0.00016933	0.022473	0.721693381	-1.38563

Table 4.7 (continued)

12691139	PAK6	p21 protein (Cdc42	0.00017 4499	0.02303	0.722982 157	-1.38316
12760883	PRKC A	protein kinase C, alpha	0.00017 5261	0.02303	0.768338 315	-1.30151
12821009	KAZA LD1	Kazal-type serine peptidase inhibitor domain 1	0.00017 6297	0.0230525	0.664442 997	-1.50502
12782531	PDK1	pyruvate dehydrogenase kinase, isozyme 1	0.00018 0959	0.023272	0.927342 699	-1.07835
12892001	KLHL9	kelch-like 9 (Drosophila)	0.00018 1304	0.023272	0.864296 765	-1.15701
12862551	GXYL T1	glucoside xylosyltransferase 1	0.00018 1488	0.023272	1.110395 523	1.1104
12901117	HEY2	hairy	0.00018 3368	0.023272	1.285266 095	1.28527
12766850	BT.624 30	angiotensin I converting enzyme	0.00018 3914	0.023272	0.877878 344	-1.13911
12866811	TAPBP L	TAP binding protein-like	0.00018 4045	0.023272	1.184940 824	1.18494
12755625	HP	haptoglobin	0.00018 4395	0.023272	0.471413 486	-2.12128
12847578	LOC61 6625	aquaporin 12B	0.00018 6353	0.023272	0.745701 034	-1.34102
12890151	SHC3	SHC (Src homology 2 domain containing) transforming protein 3	0.00018 6712	0.023272	0.509126 085	-1.96415

Table 4.7 (continued)

12684272	SLC25A36	solute carrier family 25, member 36	0.000186931	0.023272	1.106474981	1.10647
12827916	ZMYND17	zinc finger, MYND-type containing 17	0.000187572	0.023272	1.118304293	1.1183
12915057			0.000193642	0.023814	1.184233588	1.18423
12841186	SEC22B	SEC22 vesicle trafficking protein homolog B (S. cerevisiae)	0.000194389	0.023814	0.904543522	-1.10553
12749545	BCAM	basal cell adhesion molecule (Lutheran blood group)	0.000194619	0.023814	1.243713031	1.24371
12717993	SVIL	supervillin	0.000198067	0.0241252	0.704816008	-1.41881
12910551	ZBTB33	zinc finger and BTB domain containing 33	0.000199082	0.0241387	0.883080184	-1.1324
12874907	CDKL2	cyclin-dependent kinase-like 2 (CDC2-related kinase)	0.000201696	0.0243449	0.804265826	-1.24337
12687032	PTTG1IP	pituitary tumor-transforming 1 interacting protein	0.00020402	0.0243844	1.055259731	1.05526
12683071	LSAMP	neuronal growth regulator 1-like	0.000204208	0.0243844	1.356555759	1.35656
12716574	GPR158	G protein-coupled receptor 158	0.000204765	0.0243844	0.740126 71	-1.35112
12810467	GALR1	galanin receptor 1	0.000217049	0.0256058	0.630600521	-1.58579

Table 4.7 (continued)

12809257	COLEC12	collectin sub-family member 12	0.000219757	0.0256058	1.294069538	1.29407
12749084	LOC100336502	aTPase, H+	0.000219966	0.0256058	1.365702299	1.3657
12724853	EYA1	eyes absent homolog 1 (Drosophila)	0.000220297	0.0256058	1.265569671	1.26557
12801442	SLC17A1	solute carrier family 17 (sodium phosphate), member 1	0.000220419	0.0256058	0.595745188	-1.67857
12794552	GTDC2	glycosyltransferase-like domain containing 2	0.000220781	0.0256058	0.909057852	-1.10004
12865033	BCL2L14	BCL2-like 14 (apoptosis facilitator)	0.000225534	0.0257878	0.738312513	-1.35444
12773256	LRRC46	leucine rich repeat containing 46	0.000225769	0.0257878	0.782981122	-1.27717
12771117	PDK2	pyruvate dehydrogenase kinase, isozyme 2	0.000226165	0.0257878	0.848968503	-1.1779
12824808	FGFR1	fibroblast growth factor receptor 1	0.000226217	0.0257878	1.206971467	1.20697
12887654	LYSMD3	LysM, putative peptidoglycan-binding, domain containing 3	0.000230485	0.0261625	0.806659783	-1.23968
12706345	SEMA4C	sema domain, immunoglobulin domain (Ig), transmembrane domain	0.000232746	0.0263072	1.191163946	1.19116
12703330	PAIP2B	poly(A) binding protein interacting protein 2B	0.000235805	0.0265405	1.222718102	1.22272

Table 4.7 (continued)

12853653	YKT6	YKT6 v-SNARE homolog (S. cerevisiae)	0.00023 8511	0.0266211	0.846417 538	-1.18145
12885519	SAR1B	SAR1 homolog B (S. cerevisiae)	0.00023 8517	0.0266211	0.860985 312	-1.16146
12914859			0.00024 1756	0.0266288	0.864431 247	-1.15683
12782451	4-Mar	membrane-associated ring finger (C3HC4) 4, E3 ubiquitin prote	0.00024 2022	0.0266288	0.734694 477	-1.36111
12742060	SEC14 L2	SEC14-like 2 (S. cerevisiae)	0.00024 2347	0.0266288	1.324896 393	1.3249
12770437	AMZ2	archaelysin family metallopeptidase 2	0.00024 2579	0.0266288	0.894406 382	-1.11806
12769323	CAMK K1	calcium	0.00024 3731	0.0266456	1.200349 062	1.20035
12829453	TM7SF 2	transmembrane 7 superfamily member 2	0.00024 6126	0.0267976	0.732681 247	-1.36485
12893064	IL33	interleukin 33	0.00024 9169	0.0270186	0.537935 19	-1.85896
12696484	PNMA 1	paraneoplastic antigen MA1	0.00025 0658	0.0270701	0.829999 502	-1.20482
12682682	LXN	latexin	0.00025 2328	0.0270924	0.561665 225	-1.78042
12786382	AMAC R	alpha-methylacyl-CoA racemase	0.00025 2896	0.0270924	0.772194 809	-1.29501

Table 4.7 (continued)

12882320	GALNT10	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalac	0.000254175	0.0271204	1.232255521	1.23226
12842993	LMNA	lamin A	0.000255266	0.0271245	0.843106341	-1.18609
12771489	SMTNL2	smoothelin-like 2	0.000256501	0.0271245	1.243123971	1.24312
12816911	GPRC5B	G protein-coupled receptor, family C, group 5, member B	0.000257662	0.0271245	1.295618736	1.29562
12713584	CHGB	chromogranin B (secretogranin 1)	0.000261051	0.0271245	0.875013125	-1.14284
12713592	NPBWR2	neuropeptides B	0.000261347	0.0271245	0.822699937	-1.21551
12827434	USP54	ubiquitin specific peptidase 54-like	0.000261364	0.0271245	1.139607633	1.13961
12763243	FLII	flightless I homolog (Drosophila)	0.000263057	0.0271245	0.854525567	-1.17024
12771261	SGK494	uncharacterized serine	0.000263231	0.0271245	1.282812747	1.28281
12807700	SEH1L	SEH1-like (S. cerevisiae)	0.000263364	0.0271245	0.899871318	-1.11127
12914903			0.000269858	0.0276864	0.922347559	-1.08419
12857261			0.000272609	0.0278615	0.919793966	-1.0872

Table 4.7 (continued)

12797969	PLCD1	phospholipase C, delta 1	0.00027 3898	0.0278806	1.210662 547	1.21066
12850085	SEMA 3C	sema domain, immunoglobulin domain (Ig), short basic domain,	0.00027 7219	0.0278806	1.411187 613	1.41119
12773855	SP140 L	SP140 nuclear body protein-like	0.00027 7568	0.0278806	1.197123 074	1.19712
12914733			0.00027 7836	0.0278806	1.170401 026	1.1704
12782828	PLEK HM3	pleckstrin homology domain containing, family M, member 3	0.00027 9063	0.0278806	1.208707 529	1.20871
12786990	PIK3R 1	phosphoinositide-3-kinase, regulatory subunit 1 (alpha)	0.00027 9067	0.0278806	1.153558 208	1.15356
12908260	CA5B	carbonic anhydrase VB, mitochondrial	0.00028 2571	0.027969	1.219067 681	1.21907
12872493	EMCN	endomucin	0.00028 2722	0.027969	1.359140 697	1.35914
12718182	ATP5C 1	ATP synthase, H+ transporting, mitochondrial F1 complex, gamm	0.00028 3098	0.027969	0.877446 98	-1.13967
12829389	IGF2	insulin-like growth factor 2 (somatomedin A)	0.00028 6834	0.0279962	1.144147 742	1.14415
12678652	COPB2	coatomer protein complex, subunit beta 2 (beta prime)	0.00028 7375	0.0279962	0.907490 426	-1.10194
12734323	DHRS3	dehydrogenase	0.00028 8108	0.0279962	1.212640 075	1.21264

Table 4.7 (continued)

12791056	GABRG3	gamma-aminobutyric acid (GABA) A receptor, gamma 3	0.000288477	0.0279962	1.644139219	1.64414
12900549	CITED2	Cbp	0.000288621	0.0279962	1.22554126	1.22554
12755952	GOT2	glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate amin	0.000291414	0.0281648	0.910158277	-1.09871
12814236	PPP1R35	protein phosphatase 1, regulatory subunit 35	0.00029461	0.0283708	0.869860213	-1.14961
12708563			0.00030118	0.0288992	0.782870787	-1.27735
12754277	FAM65A	family with sequence similarity 65, member A	0.000306254	0.02923	0.807591359	-1.23825
12833395	CTSF	cathepsin F	0.000306819	0.02923	1.121600569	1.1216
12730119	MGC137098	uncharacterized protein MGC137098	0.000313031	0.0297006	0.808106929	-1.23746
12759391	MEIS3	Meis homeobox 3	0.000314998	0.0297006	0.752536046	-1.32884
12732959	KIF18A	kinesin family member 18A	0.000315099	0.0297006	0.782013685	-1.27875
12888569	CDC42SE2	CDC42 small effector 2	0.000317296	0.0298024	1.166652861	1.16665
12893911	APBA1	amyloid beta (A4) precursor protein-binding, family A, member	0.000320335	0.0299822	1.184337375	1.18434

Table 4.7 (continued)

12726480	MIR2318	microRNA mir-2318	0.000323937	0.0301913	0.831234466	-1.20303
12720297	TRPC4AP	transient receptor potential cation channel, subfamily C, me	0.00032673	0.0301913	0.892618049	-1.1203
12800376	PXK	PX domain containing serine	0.00032878	0.0301913	0.834898769	-1.19775
12893958	GABBR2	gamma-aminobutyric acid (GABA) B receptor, 2	0.00032872	0.0301913	1.3953404	1.39534
12897282	REV3L	REV3-like, catalytic subunit of DNA polymerase zeta (yeast)	0.00032979	0.0301913	1.147232359	1.14723
12789006	CHGA	chromogranin A (parathyroid secretory protein 1)	0.00033 0485	0.0301913	1.318871468	1.31887
12726663	CD44	CD44 molecule (Indian blood group)	0.00033 1275	0.0301913	0.809742826	-1.23496
12682117	C1H21orf91	chromosome 1 open reading frame, human C21orf91	0.00033 2146	0.0301913	0.734845646	-1.36083
12678864	HTR1F	5-hydroxytryptamine (serotonin) receptor 1F	0.00033 2755	0.0301913	0.352941592	-2.83333
12723978	MRPL33	mitochondrial ribosomal protein L33	0.00033 4872	0.0302245	0.864012995	-1.15739
12883172	HAPLN1	hyaluronan and proteoglycan link protein 1	0.00033692	0.0302245	0.732842329	-1.36455
12888990	BT.86327	---	0.00033 7588	0.0302245	1.589499133	1.5895

Table 4.7 (continued)

12863567	LOC100297468	cD24 molecule-like	0.00033 7821	0.0302245	0.579344 067	-1.72609
12793713	SPTSSA	serine palmitoyltransferase, small subunit A	0.00033 9183	0.0302245	0.893335 716	-1.1194
12725810	CTHRC1	collagen triple helix repeat containing 1	0.00033 9919	0.0302245	0.732960 501	-1.36433
12847281	UQCRH	ubiquinol-cytochrome c reductase hinge protein	0.00034 2227	0.0303286	0.896772 516	-1.11511
12828385	OGDHL	oxoglutarate dehydrogenase-like	0.00034 3961	0.030344	0.838033 303	-1.19327
12890349	ZNF462	zinc finger protein 462	0.00034 4676	0.030344	1.226116 778	1.22612
12900759	SLC18B1	solute carrier family 18, subfamily B, member 1	0.00035 1444	0.030838	1.165346 329	1.16535
12709313	IPO5	importin 5	0.00035 3896	0.0309374	0.884987 079	-1.12996
12834259	PANX1	pannexin 1	0.00035 5011	0.0309374	0.870450 806	-1.14883
12793325	FBLN5	fibulin 5	0.00035 6055	0.0309374	1.266757 62	1.26676
12725994	BAALC	brain and acute leukemia, cytoplasmic	0.00035 8274	0.030978	0.833583 408	-1.19964
12735935	SRGAP2	SLIT-ROBO Rho GTPase activating protein 2	0.00035 9732	0.030978	1.136603 873	1.1366

Table 4.7 (continued)

12867984	TENC1	tensin like C1 domain containing phosphatase (tensin 2)	0.00036111	0.030978	1.189908151	1.18991
12752761	BT.30326	---	0.00036 1226	0.030978	0.883595172	-1.13174
12720820	C1QL3	complement component 1, q subcomponent-like 3	0.00036 2329	0.030978	0.642153526	-1.55726
12870236	LPHN3	latrophilin 3	0.00036 5286	0.031131	1.481084331	1.48109
12850899	CTTNBP2	cortactin binding protein 2	0.00036 9534	0.0311323	1.173783315	1.17378
12782838	FAM126B	family with sequence similarity 126, member B	0.00037 0213	0.0311323	0.877531679	-1.13956
12720176	FLRT3	fibronectin leucine rich transmembrane protein 3	0.00037 0258	0.0311323	1.172379673	1.17238
12794461	COPG1	coatomer protein complex, subunit gamma	0.00037 049	0.0311323	0.887547706	-1.1267
12843700	SLC44A3	solute carrier family 44, member 3	0.00037 1137	0.0311323	1.231869954	1.23187
12852942	CFTR	cystic fibrosis transmembrane conductance regulator (ATP-	0.00037 3023	0.0311924	0.702740689	-1.423
12728764	SCUBE2	signal peptide, CUB domain and EGF like domain containing 2	0.00037 5739	0.0313214	1.255415549	1.25542
12861868	RERG	RAS-like, estrogen-regulated, growth inhibitor	0.00037 8144	0.031359	0.717252064	-1.39421

Table 4.7 (continued)

12696911	IGDCC4	immunoglobulin superfamily, DCC subclass, member 4	0.000378541	0.031359	1.283947574	1.28395
12843378	ATP6V0B	ATPase, H+ transporting, lysosomal 21kDa, V0 subunit b	0.000381171	0.0314791	0.865239022	-1.15575
12684999	KCNJ6	potassium inwardly-rectifying channel, subfamily J, member 6	0.000384105	0.0316235	0.671118419	-1.49005
12874253	GAR1	GAR1 ribonucleoprotein homolog (yeast)	0.000387854	0.0317025	0.776614582	-1.28764
12827902	MICU1	mitochondrial calcium uptake 1	0.000387935	0.0317025	0.922773118	-1.08369
12787557	SH3PXD2B	SH3 and PX domains 2B	0.000389192	0.0317025	1.181724396	1.18172
12762367	MAP3K3	mitogen-activated protein kinase kinase kinase 3	0.000389819	0.0317025	1.140518754	1.14052
12860901	TMTC1	transmembrane and tetratricopeptide repeat containing 1	0.0003924	0.0318053	1.287070858	1.28707
12706012	EFR3B	EFR3 homolog B (S. cerevisiae)	0.000394475	0.0318053	0.716794495	-1.3951
12886767	KXD1	KxDL motif containing 1	0.000395284	0.0318053	0.882822915	-1.13273
12803164	GNMT	glycine N-methyltransferase	0.000395853	0.0318053	0.832015975	-1.2019
12693805	RPS6KA5	ribosomal protein S6 kinase, 90kDa, polypeptide 5	0.000399962	0.032039	1.180848061	1.18085

Table 4.7 (continued)

12756690			0.000405224	0.0323633	0.789540172	-1.26656
12681327	DYRK1A	dual-specificity tyrosine-(Y)-phosphorylation regulated kinas	0.000407106	0.0324166	1.099331167	1.09933
12748294	ZNF605	zinc finger protein 605	0.000409743	0.0325294	1.119083963	1.11908
12861444	SYT10	synaptotagmin X	0.000412261	0.0326171	1.384230569	1.38423
12867796	ACVR1B	activin A receptor, type IB	0.000413292	0.0326171	1.118209255	1.11821
12911995			0.000414571	0.0326215	1.171847993	1.17185
12838286	NOTCH2	notch 2	0.000416917	0.0327096	1.201684762	1.20169
12711493	FRY	furry homolog (Drosophila)	0.000419974	0.0328111	1.190640612	1.19064
12885485	GLRX	glutaredoxin (thioltransferase)	0.000421727	0.0328111	0.836120401	-1.196
12782135	ERBB4	erb-b2 receptor tyrosine kinase 4	0.000421901	0.0328111	1.254325856	1.25433
12868475	ANKS1B	ankyrin repeat and sterile alpha motif domain containing 1B	0.000424675	0.0329308	1.225704995	1.2257
12873141	BTC	betacellulin	0.000427725	0.0330202	1.418270731	1.41827

Table 4.7 (continued)

12885399	ERAP1	endoplasmic reticulum aminopeptidase 1	0.00042 8303	0.0330202	1.251945 21	1.25195
12896756	QKI	QKI, KH domain containing, RNA binding	0.00042 9992	0.0330549	1.141169 516	1.14117
12774157	CNTN AP5	contactin associated protein-like 5	0.00043 4841	0.0333316	1.387898 084	1.3879
12899842	MOXD 1	monooxygenase, DBH-like 1	0.00043 675	0.0333433	0.625480 838	-1.59877
12858199	LRIG3	leucine-rich repeats and immunoglobulin-like domains 3	0.00043 8412	0.0333433	1.294873 853	1.29487
12866171	GOLT1 B	golgi transport 1B	0.00043 8744	0.0333433	0.869482 05	-1.15011
12812980	RASA4	RAS p21 protein activator 4	0.00044 0354	0.0333706	1.145554 105	1.14555
12790698	PPP4R 4	protein phosphatase 4, regulatory subunit 4	0.00044 1766	0.0333827	1.247242 036	1.24724
12778629	WIPF1	WAS	0.00044 4035	0.0334594	1.192497 758	1.1925
12706299	NCOA 1	nuclear receptor coactivator 1	0.00044 6813	0.0335739	1.123350 64	1.12335
12867807	CSRP2	cysteine and glycine-rich protein 2	0.00044 8135	0.0335786	1.178117 18	1.17812
12790124	ALPK3	alpha-kinase 3	0.00045 0127	0.0336334	0.798288 47	-1.25268

Table 4.7 (continued)

12815419	COG7	component of oligomeric golgi complex 7	0.00045 5987	0.0339761	0.866310 902	-1.15432
12900189	DSE	dermatan sulfate epimerase	0.00046 1485	0.03429	0.774077 686	-1.29186
12911809			0.00046 7454	0.034637	1.240011 706	1.24001
12826094	SEC24 C	SEC24 family, member C (S. cerevisiae)	0.00046 9176	0.0346683	0.891503 967	-1.1217
12869248	ERBB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avi	0.00047 2712	0.0348331	1.190114 906	1.19011
12834682	HTATI P2	HIV-1 Tat interactive protein 2, 30kDa	0.00047 662	0.0350243	0.861690 119	-1.16051
12859857	PLXN C1	plexin C1	0.00048 1822	0.0353093	1.259598 138	1.2596
12744300	FBXW 7	F-box and WD repeat domain containing 7	0.00048 4596	0.0354153	0.870708 496	-1.14849
12889697	RUSC2	RUN and SH3 domain containing 2	0.00048 6642	0.0354677	0.838391 629	-1.19276
12865601	MARS	methionyl-tRNA synthetase	0.00049 0565	0.0354978	0.866626 224	-1.1539
12850206	AGK	acylglycerol kinase	0.00049 0801	0.0354978	0.877454 679	-1.13966
12907940	PDK3	pyruvate dehydrogenase kinase, isozyme 3	0.00049 2327	0.0354978	0.863490 748	-1.15809

Table 4.7 (continued)

12881048	C7H5orf30	UNC119-binding protein C5orf30 homolog	0.000492818	0.0354978	0.853890753	-1.17111
12875407	RRH	retinal pigment epithelium-derived rhodopsin homolog	0.000494206	0.0354978	1.679247026	1.67925
12804713	RPS4Y1	ribosomal protein S4, Y-linked 1	0.00049504	0.0354978	0.83037857	-1.20427
12786020	C1QTNF3	C1q and tumor necrosis factor related protein 3	0.000497789	0.0355992	0.548380905	-1.82355
12772050	KIF19	kinesin family member 19	0.000501395	0.0357612	1.367467136	1.36747
12862410	PARVB	parvin, beta	0.000503447	0.035779	0.525041872	-1.90461
12712868	MRP63	mitochondrial ribosomal protein 63	0.000504327	0.035779	0.850918993	-1.1752
12850925	NACAD	NAC alpha domain containing	0.000508742	0.0358703	0.843198759	-1.18596
12842417	LOC100298793	cytochrome P450, family 2, subfamily J, polypeptide 2-l	0.000511358	0.0358703	1.419617697	1.41962
12851927	NME2	non-metastatic cells 2, protein (NM23B) expressed in	0.000511441	0.0358703	0.842829209	-1.18648
12711284	GAS6	growth arrest-specific 6	0.000512288	0.0358703	1.225511222	1.22551
12894461	CHRNA2	cholinergic receptor, nicotinic, alpha 2 (neuronal)	0.000512337	0.0358703	0.635849177	-1.5727

Table 4.7 (continued)

12914639			0.00051 4411	0.0359212	1.155110 556	1.15511
12892374	LOC10 029526 3	uncharacterized LOC100295263	0.00051 6545	0.0359281	1.114031 11	1.11403
12746149	SLC24 A6	solute carrier family 24 (sodium	0.00051 7204	0.0359281	1.179477 562	1.17948
12860410	EFCA B4B	EF-hand calcium binding domain 4B	0.00052 3419	0.0362076	1.277811 569	1.27781
12740926	DUSP1 0	dual specificity phosphatase 10	0.00052 7983	0.0362076	1.221833 679	1.22183
12857795	AGAP 2	ArfGAP with GTPase domain, ankyrin repeat and PH domain 2	0.00052 8276	0.0362076	1.240577 812	1.24058
12771875	SRCIN 1	SRC kinase signaling inhibitor 1	0.00052 8292	0.0362076	1.208958 869	1.20896
12898982	MGC1 27538	uncharacterized protein MGC127538	0.00052 9202	0.0362076	0.669841 716	-1.49289
12710496	DCLK 1	doublecortin-like kinase 1	0.00052 9371	0.0362076	0.753698 777	-1.32679
12765386	SLC39 A11	solute carrier family 39 (metal ion transporter), member 11	0.00053 1096	0.0362327	0.822882 723	-1.21524
12723165	ADCY 8	adenylate cyclase 8 (brain)	0.00053 4084	0.0363436	0.791114 205	-1.26404
12901045	MAP3 K5	mitogen-activated protein kinase kinase kinase 5	0.00053 7613	0.0364217	1.298559 638	1.29856

Table 4.7 (continued)

12740562	EPRS	glutamyl-prolyl-tRNA synthetase	0.00053 7963	0.0364217	0.890749 566	-1.12265
12822071	MYOF	myoferlin	0.00054 2297	0.0366068	1.271674 095	1.27167
12698500	CNGA 3	cyclic nucleotide gated channel alpha 3	0.00054 3441	0.0366068	0.750632 408	-1.33221
12710994	SERT M1	serine-rich and transmembrane domain containing 1	0.00054 8783	0.0367467	1.646334 436	1.64633
12822230	ALDH 18A1	aldehyde dehydrogenase 18 family, member A1	0.00054 9551	0.0367467	0.890376 807	-1.12312
12728517	LDLR AD3	low density lipoprotein receptor class A domain containing 3	0.00055 2262	0.0367467	1.193025 573	1.19303
12850342	TNS3	tensin 3	0.00055 2327	0.0367467	1.132020 793	1.13202
12767297	ARRB 2	arrestin, beta 2	0.00055 2406	0.0367467	1.205350 793	1.20535
12692077	RAD51 B	RAD51 homolog B (S. cerevisiae)	0.00055 7082	0.0368933	1.175414 098	1.17541
12811667	ACTL6 B	actin-like 6B	0.00055 7477	0.0368933	0.885206 43	-1.12968
12784195	WDFY 1	WD repeat and FYVE domain containing 1	0.00055 8759	0.0368933	1.167194 822	1.16719
12856701	CS	citrate synthase	0.00056 2572	0.0370533	0.908694 388	-1.10048

Table 4.7 (continued)

12695573	LOC527711	spectrin beta chain, erythrocyte-like	0.000569403	0.0374109	0.835470746	-1.19693
12721237	JAG1	jagged 1	0.000571214	0.0374377	1.236745183	1.23675
12699180	CRIM1	cysteine rich transmembrane BMP regulator 1 (chordin-like)	0.000575076	0.0375984	1.227322155	1.22732
12755154			0.000578463	0.037616	0.816526496	-1.2247
12883643	SLCO4C1	solute carrier organic anion transporter family, member 4C1	0.000579234	0.037616	1.512333076	1.51233
12786303			0.000580875	0.037616	0.86613082	-1.15456
12857841	AMDHD1	amidohydrolase domain containing 1	0.000580986	0.037616	1.271539886	1.27154
12692448	B2M	beta-2-microglobulin	0.000589776	0.0380545	1.232003509	1.232
12753406	SYT5	synaptotagmin V	0.000590611	0.0380545	0.881694264	-1.13418
12896514	ESR1	estrogen receptor 1	0.000595193	0.0382573	1.23512447	1.23512
12759210	U2AF1L4	U2 small nuclear RNA auxiliary factor 1-like 4	0.000598874	0.0383285	1.111858527	1.11186
12879784	PRELID1	PRELI domain containing 1	0.000599175	0.0383285	0.903481113	-1.10683

Table 4.7 (continued)

12788870	MIR329B	microRNA mir-329b	0.000604208	0.0384602	0.644932443	-1.55055
12773652	LEPREL4	leprecan-like 4	0.00060462	0.0384602	0.87152805	-1.14741
12899728	PGM3	phosphoglucomutase 3	0.000605559	0.0384602	0.862887221	-1.1589
12875625	SMIM14	small integral membrane protein 14	0.000612085	0.0386216	0.895070845	-1.11723
12846438	UAP1	UDP-N-acteylglucosamine pyrophosphorylase 1	0.000613386	0.0386216	0.840830741	-1.1893
12880280	BT.34956	---	0.00061365	0.0386216	0.81617329	-1.22523
12854454	RAPGEF5	Rap guanine nucleotide exchange factor (GEF) 5	0.000613892	0.0386216	1.30135471	1.30136
12725367	GRHL2	grainyhead-like 2 (Drosophila)	0.000621379	0.0388747	1.251741485	1.25174
12880370	PCDHGB4	protocadherin gamma subfamily B, 4	0.000621625	0.0388747	1.152806854	1.15281
12700679	NBAS	neuroblastoma amplified sequence	0.000622945	0.0388747	0.882005327	-1.13378
12675621			0.000624917	0.0388747	1.576764794	1.57677
12696845	SLC8A3	solute carrier family 8 (sodium	0.000625201	0.0388747	0.655849522	-1.52474

Table 4.7 (continued)

12807569	ST8SIA3	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransfe	0.000629731	0.039007	1.140526558	1.14053
12864912	SHMT2	serine hydroxymethyltransferase 2 (mitochondrial)	0.000630253	0.039007	0.837527952	-1.19399
12752645	SLC7A6	solute carrier family 7 (amino acid transporter light chain,	0.00063368	0.0391193	0.845437176	-1.18282
12686282	FAM43A	family with sequence similarity 43, member A	0.000635002	0.0391193	1.310209018	1.31021
12779984	MIR2356	microRNA mir-2356	0.000641561	0.0394323	1.375402305	1.3754
12831806	SYTL2	synaptotagmin-like 2	0.000644972	0.0395509	0.616614049	-1.62176
12857257	SLC25A3	solute carrier family 25 (mitochondrial carrier; phosphate carr	0.000648197	0.0395957	0.912508669	-1.09588
12714175	PRKCQ	protein kinase C, theta	0.000649741	0.0395957	0.680804711	-1.46885
12834738	ZBTB44	zinc finger and BTB domain containing 44	0.000650209	0.0395957	1.095670676	1.09567
12898481	EYA4	eyes absent homolog 4 (Drosophila)	0.000651164	0.0395957	1.30495832	1.30496
12824728	MIR2400	microRNA mir-2400	0.000655673	0.0397483	1.079059449	1.07906
12683496	ITGB5	integrin, beta 5	0.000657132	0.0397483	1.246624763	1.24662

Table 4.7 (continued)

12709172	MCF2L	MCF.2 cell line derived transforming sequence-like	0.000659204	0.0397834	1.147817024	1.14782
12789506	PDE8A	phosphodiesterase 8A	0.00066136	0.0398234	0.855073579	-1.16949
12813668	CALN1	calneuron 1	0.00066 3369	0.0398544	1.292863265	1.29286
12874507	NSG1	neuron specific gene family member 1	0.00066 7118	0.0399896	1.246686929	1.24669
12811457	DNASE1	deoxyribonuclease I	0.00066 9711	0.040055	1.104228089	1.10423
12765442	YPEL2	yippee-like 2 (Drosophila)	0.00067 5232	0.0402949	0.797384579	-1.2541
12705319	CHST10	carbohydrate sulfotransferase 10	0.00067 6835	0.0403004	0.872965989	-1.14552
12906927	TBC1D8B	TBC1 domain family, member 8B (with GRAM domain)	0.00067 9038	0.0403415	0.870958751	-1.14816
12803392	SLC39A7	solute carrier family 39 (zinc transporter), member 7	0.00068 3522	0.0405177	0.878757788	-1.13797
12851829	AMPH	amphiphysin	0.00068 7146	0.0405511	1.255626777	1.25563
12841921	LOC100848786	lipid phosphate phosphatase-related protein type 5-like	0.00068 7415	0.0405511	1.290663982	1.29066
12890580	CNTRL	centriolin	0.00068 8646	0.0405511	1.162593327	1.16259

Table 4.7 (continued)

12859128	C1R	complement component 1, r subcomponent	0.00069 0455	0.040568	1.266630 863	1.26663
12863934	BTG1	B-cell translocation gene 1, anti-proliferative	0.00069 5771	0.0407905	1.218942 86	1.21894
12879860	RASG RF2	Ras protein-specific guanine nucleotide-releasing factor 2	0.00070 411	0.0411889	1.155657 87	1.15566
12698459	POMC	proopiomelanocortin	0.00071 1262	0.0415162	0.797441 807	-1.25401
12731200	LGR4	leucine-rich repeat containing G protein-coupled receptor 4	0.00071 4431	0.0415858	1.135924 756	1.13593
12711615	COG6	component of oligomeric golgi complex 6	0.00071 6027	0.0415858	0.909173 561	-1.0999
12845308	TCTE X1D1	Tctex1 domain containing 1	0.00071 7131	0.0415858	1.402495 039	1.4025
12884927	PJA2	praja ring finger 2	0.00072 1913	0.0416887	0.919438 775	-1.08762
12750642	TERF2 IP	telomeric repeat binding factor 2, interacting protein	0.00072 2031	0.0416887	0.845408 586	-1.18286
12803365			0.00073 0378	0.0420796	1.115262 365	1.11526
12676495			0.00073 4621	0.0422328	1.381036 164	1.38104
12691075	SV2C	synaptic vesicle glycoprotein 2C	0.00074 0752	0.0424937	0.453570 278	-2.20473

Table 4.7 (continued)

12900080	TCP1	t-complex 1	0.00074 5803	0.0426916	0.857728 563	-1.16587
12780180	PKP4	plakophilin 4	0.00075 7244	0.0431826	1.084579 877	1.08458
12715823	ARMC 4	armadillo repeat containing 4	0.00075 7617	0.0431826	1.168029 173	1.16803
12861001	SOX5	SRY (sex determining region Y)-box 5	0.00075 9798	0.0432145	1.193276 128	1.19328
12746075	RFC5	replication factor C (activator 1) 5, 36.5kDa	0.00076 2194	0.0432236	1.077600 141	1.0776
12801783	LOC51 2672	major histocompatibility complex, class I	0.00076 3198	0.0432236	1.198532 038	1.19853
12812837	ERCC4	excision repair cross-complementing rodent repair deficiency,	0.00076 984	0.0435074	1.096765 419	1.09677
12695501	SCG3	secretogranin III	0.00077 4345	0.0436695	0.776934 372	-1.28711
12752141	MTSS1 L	metastasis suppressor 1-like	0.00077 7449	0.0437029	0.815281 639	-1.22657
12826866	LRRT M3	leucine rich repeat transmembrane neuronal 3	0.00077 9829	0.0437029	1.573853 88	1.57385
12782645	PADI6	peptidyl arginine deiminase, type VI	0.00077 9854	0.0437029	0.741383 273	-1.34883
12688919	LYSM D2	LysM, putative peptidoglycan-binding, domain containing 2	0.00078 5376	0.0437927	0.785040 273	-1.27382

Table 4.7 (continued)

12904088	ALG13	asparagine-linked glycosylation 13 homolog (S. cerevisiae)	0.00078 7521	0.0437927	1.221019 111	1.22102
12840703	C3H1o rf111	chromosome 3 open reading frame, human C1orf111	0.00078 7783	0.0437927	0.766618 37	-1.30443
12792023	FES	feline sarcoma oncogene	0.00079 0434	0.0437927	1.212207 903	1.21221
12722327	TSTA3	tissue specific transplantation antigen P35B	0.00079 4299	0.0437927	0.843355 204	-1.18574
12835758	SV2A	synaptic vesicle glycoprotein 2A	0.00079 551	0.0437927	1.194972 988	1.19497
12723553	DEPT OR	DEP domain containing MTOR-interacting protein	0.00079 5644	0.0437927	1.159042 677	1.15904
12914751			0.00079 6285	0.0437927	0.751738 395	-1.33025
12908846	GABR E	gamma-aminobutyric acid (GABA) A receptor, epsilon	0.00079 8033	0.0437927	1.281408 935	1.28141
12716263	RPN2	ribophorin II	0.00079 843	0.0437927	0.885927 921	-1.12876
12911099			0.00080 2964	0.0437927	1.160710 866	1.16071
12876698	C3	complement component 3	0.00080 3048	0.0437927	1.471232 982	1.47123
12740723	LOC53 9953	denticleless protein homolog	0.00080 336	0.0437927	0.738029 167	-1.35496

Table 4.7 (continued)

12849407	MEST	mesoderm specific transcript homolog (mouse)	0.00080444	0.0437927	0.843540169	-1.18548
12715842	TTPAL	tocopherol (alpha) transfer protein-like	0.00081065	0.0439839	0.888754588	-1.12517
12825112	WHSC1L1	Wolf-Hirschhorn syndrome candidate 1-like 1	0.00081249	0.0439839	1.069198529	1.0692
12828947	JMJD1C	jumonji domain containing 1C	0.00081765	0.0442412	1.10987545	1.10988
12678271	TMEM207	transmembrane protein 207	0.00083465	0.0448318	0.863490748	-1.15809
12902219	GPRASP1	G protein-coupled receptor associated sorting protein 1	0.00083572	0.0448318	0.837773533	-1.19364
12824828	SFRP1	secreted frizzled-related protein 1	0.00083937	0.0448318	1.233955494	1.23395
12818938	GRK5	G protein-coupled receptor kinase 5	0.00084032	0.0448318	1.185206256	1.18521
12873401	BMPR1B	bone morphogenetic protein receptor, type IB	0.00084115	0.0448318	1.246388589	1.24639
12873854	PLAC8	placenta-specific 8	0.00084159	0.0448318	1.776075902	1.77608
12808035	CHST9	carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 9	0.00084185	0.0448318	0.825157399	-1.21189
12790928	LRFN5	leucine rich repeat and fibronectin type III domain containing	0.00084201	0.0448318	1.44951905	1.44952

Table 4.7 (continued)

12875951	C6H4orf32	chromosome 6 open reading frame, human C4orf32	0.000845028	0.0449026	0.715358752	-1.3979
12861802	PARP11	poly (ADP-ribose) polymerase family, member 11	0.000847815	0.0449612	1.109924725	1.10992
12897014	FRK	fyn-related kinase	0.000849767	0.0449753	1.359885552	1.35989
12896683	BCKDHB	branched chain keto acid dehydrogenase E1, beta polypep	0.00085525	0.0449922	0.8702462 8	-1.1491
12786321	ESM1	endothelial cell-specific molecule 1	0.000856092	0.0449922	0.5561951 8	-1.79793
12824753	PLAT	plasminogen activator, tissue	0.000856745	0.0449922	1.2661497 4	1.26615
12825144	PPP1R3B	protein phosphatase 1, regulatory subunit 3B	0.000856834	0.0449922	0.7757169 56	-1.28913
12698660	EPAS1	endothelial PAS domain protein 1	0.000859307	0.0450334	1.2382336 84	1.23823
12842746	ATP1A1	ATPase, Na+	0.000871202	0.0455673	0.8909717 83	-1.12237
12692159	REEP5	receptor accessory protein 5	0.000873341	0.0455898	0.9323922 39	-1.07251
12787195	SLC30A5	solute carrier family 30 (zinc transporter), member 5	0.000876799	0.0456334	0.9112945 85	-1.09734
12915041			0.000877599	0.0456334	0.7639185 97	-1.30904

Table 4.7 (continued)

12749816	GAS8	growth arrest-specific 8	0.00089 2365	0.0462828	1.171628 317	1.17163
12892286	STOM	stomatin	0.00089 4615	0.0462828	1.156473 127	1.15647
12832646	SRPR	signal recognition particle receptor (docking protein)	0.00089 5292	0.0462828	0.862522 533	-1.15939
12875956	PLAC8	placenta-specific 8	0.00089 9949	0.0464335	1.357144 893	1.35714
12829383	GNG3	guanine nucleotide binding protein (G protein), gamma 3	0.00090 3666	0.0465251	0.726738 905	-1.37601
12741898	CPE	carboxypeptidase E	0.00090 5212	0.0465251	0.905764 284	-1.10404
12897969	BAI3	brain-specific angiogenesis inhibitor 3	0.00090 769	0.0465627	1.217785 02	1.21778
12915157			0.00091 4302	0.046758	0.823614 68	-1.21416
12807710	FAM59 A	family with sequence similarity 59, member A	0.00091 5002	0.046758	1.113502 667	1.1135
12915155			0.00091 9174	0.0468174	0.845129 939	-1.18325
12788800	MIR54 4A	microRNA mir-544a	0.00091 9675	0.0468174	0.616431 601	-1.62224
12747065	MSI1	musashi homolog 1 (Drosophila)	0.00092 6472	0.0469521	1.245961 527	1.24596

Table 4.7 (continued)

12910177	MID1IP1	MID1 interacting protein 1 (gastrulation specific G12 homolo	0.000931189	0.0469521	1.144915374	1.14492
12830490	LOC512612	histone H2B type 1-like	0.00093122	0.0469521	1.153970871	1.15397
12794684	CSPG5	chondroitin sulfate proteoglycan 5 (neuroglycan C)	0.000931634	0.0469521	1.378454752	1.37846
12787836	PELO	pelota homolog (Drosophila)	0.000932739	0.0469521	0.887839266	-1.12633
12682282	APOD	apolipoprotein D	0.000934393	0.0469521	1.471865295	1.47186
12836105	GNG12	guanine nucleotide binding protein (G protein), gamma 12	0.000934642	0.0469521	0.817795224	-1.2228
12834462	PITPNM1	phosphatidylinositol transfer protein, membrane-associated 1	0.000938923	0.0470785	0.827876249	-1.20791
12914259			0.000944074	0.0471625	0.793197538	-1.26072
12814004	TMEM204	transmembrane protein 204	0.000944133	0.0471625	1.160667755	1.16067
12679678	PLCL2	phospholipase C-like 2	0.000948677	0.0473009	1.155484275	1.15548
12765359	FAM104A	family with sequence similarity 104, member A	0.000952402	0.0473137	0.900495272	-1.1105
12691043	TTLL5	tubulin tyrosine ligase-like family, member 5	0.000952481	0.0473137	1.13304048	1.13304

Table 4.7 (continued)

12839347	WLS	wntless homolog (Drosophila)	0.000960055	0.0476013	1.161903058	1.1619
12761717	MSI2	musashi homolog 2 (Drosophila)	0.000968534	0.0479326	0.942871421	-1.06059
12729022	MADD	MAP-kinase activating death domain	0.000972831	0.0480561	0.872638422	-1.14595
12875914	ABLIM2	actin binding LIM protein family member 2	0.0009844	0.0485377	1.292654362	1.29265
12700884	CCDC85A	coiled-coil domain containing 85A	0.000995188	0.048979	1.252536386	1.25254

Table 4.8. List of selected genes involved in prolactin or POMC/ACTH expression expressed by pituitaries collected from steers grazing high- (HE, n = 8) or low- (LE, n = 8) endophyte-infected forages.

Transcript ID	Gene Symbol	Gene_assignment	P-value	False Discovery Rate	Ratio (HE vs. LE)	Fold-Change (HE vs. LE)
12730735	DRD2	Dopamine receptor D2	5.55E-07	0.00148011	0.569216758	-1.7568
12804415	PRL	Prolactin	6.12E-06	0.00411905	0.814617496	-1.22757
12785083	PRLR	Prolactin receptor	8.86E-05	0.0163351	0.762951095	-1.3107
12683485	POU1F1	POU class 1 homeobox 1	0.00350045	0.0919041	0.769538585	-1.29948
12829370	GAL	Galanin/GMAP prepropeptide	0.00857877	0.143484	0.745089858	-1.34212
12896608	VIP	Vasoactive intestinal peptide	0.00330761	0.0891485	0.568424044	-1.75925
12698459	POMC	Proopiomelanocortin	0.000711262	0.0415162	0.797441807	-1.25401
12883237	PCSK1	Proprotein convertase subtilisin/kexin type 1	3.55E-06	0.00326731	0.580548154	-1.72251

Table 4.8 (continued)

12766978	GH1	Growth Hormone 1	0.72773	0.91774	1.005162515	1.00516
12842660	TSHB	Thyroid stimulating hormone beta	0.999161	0.999934	1.000078006	1.00008
12844718	TBX19	T-Box 19	0.104204	0.458935	0.881026219	-1.13504
12778522	NEUROD 1	Neuronal differentiation 1	0.177765	0.56633	1.188588599	1.18859
12883226	NR3C1	Nuclear receptor subfamily 3 group C member 1	0.112395	0.473489	1.137451971	1.13745
12760857	CRHR1	Corticotropin releasing hormone receptor 1	0.106139	0.461876	1.19399706	1.194
12850526	CRHR2	Corticotropin releasing hormone receptor 2	0.724778	0.916408	1.028423571	1.02842

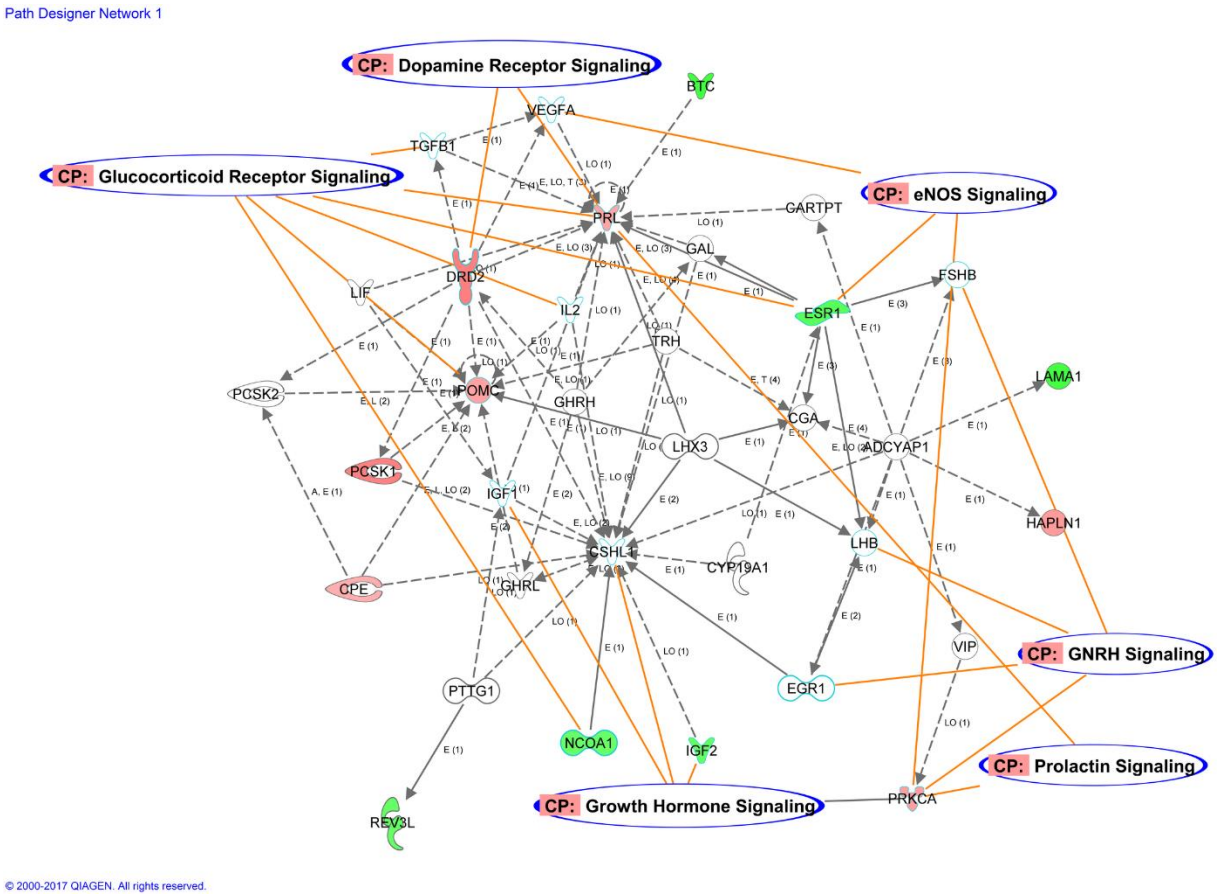

Figure 4.1. Canonical pathway network analysis. The red or green coloring represents down- or up-regulation, respectively, whereas no color indicates the molecule was added from the Ingenuity Knowledge Base (Ingenuity Pathway, Ingenuity Systems, Inc., Redwood City, CA). The intensity of the node color (light to dark) proportionally indicates the degree of differential expression. Straight lines represent binding only, whereas arrowheads symbolize action-on. A crosshead bar signifies inhibition. Labels of interaction or relationship: A = Activation, CP = Canonical Pathway, E = Expression (includes metabolism or synthesis for chemicals), I = Inhibition, LO = Localization. The number in parenthesis for each interaction indicates the number of published references in the Ingenuity Knowledge Base that support the particular interaction.

***ACTB*:**

GAGCGGGAAATCGTCCGTGACATCAAGGAGAAGCTCTGCTACGTGGCCCTGG
ACTTCGAGCAGGAGATGGCCACCGCGGCCTCCAGCTCCTCCCTGGAGAAGAG
CTACGAGCTTCCTGACGGGCAGGTCATCACCATCGGCAATGAGCGGTTCCGC
TGCCCTGAGGCTCTCTTCCAGCCTTCCTTCCTGGGCATGGAATCCTGCGGCAT
TCACGAAACTACCTTCAATTCCATCATGAAGTGTGACGTCGACATCCGCAAG
GACCTCTACACCAACAC

***PPIA*:**

GGCAAGTCCAATTATGGCGAGAAATTTGATGATGAGAATTTCATTTTGAAGC
ATACAGGTCCTGGCATCTTGTCCATGGCAAATGCTGGCCCCAACACAAATGG
TTCCCAGTTTTTCATTTGCACTGCCAAGACTGAGTGGTTGGATGGCAAGCACG
TGGTCTTTGGCAAGGTGAAAGAGGGCATGAATATTGTGGAAGCCATGGAGCG
CTTTGGGTCCAGGAATGGCAAGACCAGCAA

***UBC*:**

TAGGGGTGGGTTAGAGTTCAAGGTTTTTGTTCTACCAGATGTTTTAGTAGTAA
TCTGGAGGTAAGAAATGTCAAGAAAACATGGCCTTAATTAGAACTGTAGTGG
GTGAGTATAAATAAAAAATTTGGAGGTTGTAGTTAGAATTCTCCATATGTAC
ACTCATATGTAGATCTACTTATAAGCTACTGATTTTTAAAAGCACACGTTTGG
GAGTTGTGCTTAAGAGTGGGAAAGTTTCTGGAATACCAGCAGGGAGGT

***DRD2*:**

CGACCTTTCTCTGGGGCTTTGGGGCTCTGCGGCTGCGGGGCCAGTATCGAGGC
TCGGAGGCCTGGTTTTCACAGGCCATGCCGGAGCTGGTGGTGGGGAGGAGTG
GACAGTCACAGCCACCCAGGGCCCACACCTGAGAAGCCAGAGCTCTGGCCAC
GACCCCAGGCAGTGTCAAGCCTGGGAGACCCGCGTACACCCCAGGTCTGGAT
GGACCCCAGAGAAGCAGAAGCCCAA

***PRL*:**

AGAACAAGCCCAACAGACCCACCATGAAGTCCTTATGAGCTTGATTCTTGGG
TTGCTGCGCTCCTGGAATGACCCTCTGTATCACCTAGTCACCGAGGTGCGGGG
TATGAAAGGAGCCCCAGATGCTATCCTATCGAGGGCCATAGAGATTGAGGAA
GAAAACAAACGACTTCTGGAAGGCATGGAGATGATATTTGGCCAGGTTATTC
CGAGCGCCAAAGAGACTGAGCCCTACCCTGTGTGGTCAGGACT

***s-PRLR*:**

GCCATCCTTTCTGCTGTCATCTGTTTGATTATGGTCTGGGCAGTGGCTTTGAG
GGCTATAGCATGGTGACCTGCATCCTCCCACCAGTTCCAGGGCCAAAAATAA
AAGGATTTGATGTTCATCTGCTGGAGATATCACAGCCTTCTCGCCT

***l-PRLR*:**

CCATCCTTTCTGCTGTCATCTGTTTGATTATGGTCTGGGCAGTGGCTTTGAAG
GGCTATAGCATGGTGACCTGCATCCTCCCACCAGTTCCAGGGCCAAAAATAA
AAGGATTTGATGTTCATCTGCTGGAGAAGGG

***POU1F1 (Pit-1)*:**

AAGCAAGAGTGTTGAAGTTTGGTTTGATTTTTCTCTTTGACATGAAAAATAAG
TATCTTGTTTCATCACACTATGAAGAAAAGCAAGGCCAGTGAAAGTGTAGAA
ATAAATTTATTGAGAAGGTAAATAATGAGAGAATAAAATATATAGGGAAAGT
TTCTACACAATGTGGCATAGGTGTGAAGTGGTGAAATGATTCTTTTTAATGTA
TCCAGATTTTTTCCTGCTGTGCTATATACTGTAGTAATTATTCATGAATCATTT
TTACAACCTAATATAAGTGTAGCCAGAGCATTCGCACACACCGTTCTTTCTAG
TGAATAGCAAGCAATTGCTAGATGAACAATTTAATGTGATAAAAATTATCTA
CTTATATTAATGTCAAGGCTGGCTAAAGAGCA

***GAL*:**

CACCGGTGAAGGAGAAGAGAGGCTGGACCCTGAACAGCGCTGGGTACCTTCT
CGGACCACATGCGCTCGACAGCCACAGGTCATTTCAAGACAAGCATGGCCTC
GCCGGCAAGCGGGAACTCGAGCCTGAAGACGAAGCCCGGCCAGGAAGCTTT
GACAGACCACTGGCGGAGAACAACGTCGTGCGCACGATAATCGAGTTTCTGA
CTTTCCTGCATCTCAAAGACGCC

***VIP*:**

CTGGTTCAGCTGTAAGGGCAAGAGAACTCGTGAAGACTGTCGACTCCCAGGA
CTTCAACACCTGAGACAGCTCTCATAATCTCAACAGAAAGCTCTCAAAGAAC
ACTATTCGGCAAAGTCCTGCAATGGAAACAAGAAGTAAGCCCCAGCTTCTTG
TGTTCCTGACGCTGTTCAGCGTGCTCTTCTCCCAGACCTTGGCGTGGCCTCTTT
TTGGAGCACCTTCGGCTCTGAGGATGGGGGACAGAATACCATTTGAAGGAGC
GAATGAACCTGATCAAGTTTCGTTAAAAGCAGACACTGACATTTTACAAGAT
GCGCTGGCTGA

***POMC*:**

AGCTTCCCCCTGACAGAGCCTCAGCCTGCCTGGAAGATGCCGAGACTGTGCA
GCAGTCGTTCGGGCGCCCTGCTGCTGGCCTTGCTGCTTCAGGCCTCCATGGAA
GTGCGTGGTTGGTGCCTGGAGAGCAGCCAGTGTCAGGACCTCACCACGGAAA
GTAACCTGCTGGCGTGCATCCGGGCCTGCAAGCCCGACCTCTCCGCCGAGAC
GCCGGTGTTCCCCGGCAACGGCGACGAGCAGCCGCTGACTGAGAACCCCCGG
AAGTACGTCATGGGCCATTTCCGCTGGGACCGCTTCGGCCGTCGGAATGGTA
GCAG

***PCSK1*:**

TGATCGTGTGATATGGGCGGAACAACAGTATGAAAAAGAAAGAAGTAAACG
TTCAGTTCTAAGAGACTCAGCACTAGATCTCTTCAATGATCCGATGTGGAATC
AGCAGTGGTACTTGCAAGATACAAGGATGACTGCAACCCTGCCCAAGCTGGA
TCTCCATGTGATACCTGTTTGGCAAAAAGGCATCACAGGCAAAGGTGTTGTT
ATCACTGTATTGGATGATGGCTTGGAGTGGAATCACACAGACATCTATGCCA
ACTATGATCCGGAGGCC

GH1:

CCCAGCAGAAAANCAGACTGGAGCTGCTTCGCATCTCaCTGCTCCTCATCCAG
TCGTGGCTTGGGCCCCTGCAGTTCCTCAGCAGAGTCTTCACCAACAGCTTGGT
GTTTGGCACCTCGGACCGTGTCTATGAGAAGCTGAAGGACCTGGAGGAAGGC
ATCCTGGCCCTGATGCGGGAGCTGGAAGATGGCACCCCCCGGGCTGGGCAGA
TCCTCAAGCAGACCTATGACAAATTTGACACAAACATGCNCAGTGACGA

TSHB:

TTTTTGGCCTTGCATGTGGACAAGCAATGTCTTTTTGTATtCcaACTGAGTATAT
GATGCATGTCGAAAGGAAAGAATGTGCTTACTGCCTAACCATCAACACCACC
GTCTGTGCTGGATATTGTATGACACGGGATGTCAACGGCAAGCTGTTTCTTCC
CAAATATGCCCTGTCTCAGGATGTCTGTACATACAGAGACTTCATGTACAAG
ACTGCAGAAATACCAGGATGCCCACGCCTGGTTACTCCT

TBX19 (Tpit):

CCATGCCATGCTGGCAACAAAAGTTCGTTGAGTACCTTCCTTGTGcCCTGACC
CTTACTCTCAAATGATTCTAGAAGGCTGCCCCTCCTGTTCTGTGGACATAGTG
TAAAGCTGGTAAGTGAGCTAAAAATTTACCTAGCCTAAGGCTACTGCATCAT
AACTTTGTACAGTCCTAAAGGTATGTGTAGCCACAGAGCTTCTGCTTTGAGAC
CAAGAGAGGGTGTAAGGCCATGGAGTGAAGTTAACAGAAGTAGAGTTAATT
ACATGTTATAGCACTGAGAATTGAGGAAATGAAATCATGTTGCTCCTTGSTCA
CAAAC

NEUROD1:

GGACAGCTCCCCAGTCTTCCACGTCAAGCCGCCGCCTCACGCCTACAGCGCA
GCGCTGGAACCTTTCTTTGAGAGCCCTCTGACTGACTGCACCAGCCCTTCCTT
TGACGGACCCCTCAGCCCGCCGCTCAGCATCAATGGCAACTTCTCTTTCAAAC
ACGAACCGTCCGCCGAGTTTGAGAAAAATTATGCCTTtACCATGCACTaTcCTG
CAGCGACCCTGGCAGGGGCCCAAAGCCMSGGGATCAGTC

NR3C1 (Glucocorticoid receptor):

AAAGAGCAGTGGGAGGACAGCACAATTATCTTTGTGCTGGAAGAAATGATTG
TATCATTGATAAAATTCGAAGAAAAAACTGCCCAGCATGCCGCTATAGAAAA
TGCCTTCAAGCTGGAATGAACCTGGAAGCTCGAAAAACAAAGAAAAAGATA
AAAGGAATTCAGCAGGCCACTACGAGGAGTCTCGCANAGAAACATCTGAAA
ATCCTGCTAACAAAACAATAGTTCCTGCAACATTACCACAACTCCCCGACGC
T

CRHR1:

NNAGCAAGGNTCACTACCACATCGCTGTCATCATCAACTACCTAGGCCACTG
CATCTCCCTGGCGGCCCTCCTGGTGGCCTTTGTCCTCTTTCTGCGGCTCAGGA
GCATCCGGTGCCTGAGAAACATCATCCACTGGAACCTCATCTCAGCCTTCATC
CTGCGCAATGCCACGTGGTTCGTGGTCCAGCTCACCATGAGCCCCGAAGTCC
ATCAGAGCAACGTGGGCTGGTGCAGGCTGGTGACAGCCGCCTACAACTACTT
CCACGTGACCAACTTCTTCTGGATGTTCGGTGAGGGCTGCTACCTGCACACGG
CCATCGTGCTCACGTCTACCACAGACC

CRHR2:

GCTGGTTTTGGAGGCTGGGGGCTGCCCCTGCACCCCGAGGGTCCCTACTCCTA
CTGCAACACGACCTTGGACCAGATCGGGACGTGCTGGCCCCGGAGCTCGGCC
GGAGCCCTGGTGGAGAGGCCGTGCCCCGAGTACTTCAACGGTGTCAAGTACA
ACACGACCCGGAATGCCTACCGAGAGTGCTTGGAGAATGGGACGTGGGCCTC
GCGGATCAACTACTCACA

Figure 4.2. The sequences of the real-time RT-PCR products (5’ to 3’ orientation).

Within a sequence, underlined nucleotides indicate the forward and reverse primer

positions.

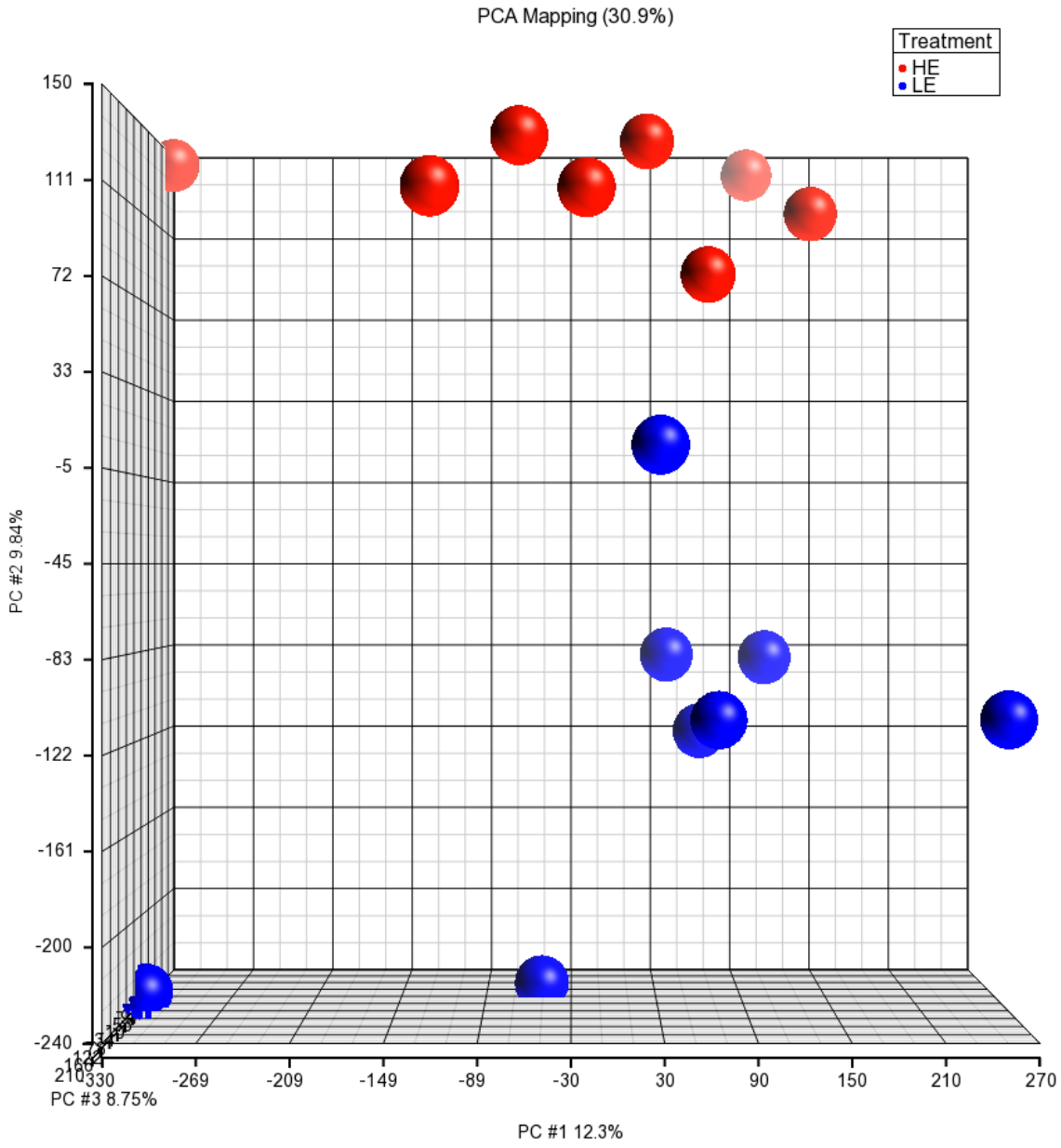

Figure 4.3. Principle component analysis of microarray transcriptome analysis of 16 pituitary samples from steers grazing high- (HE, n = 8, red dots) or low- (LE, n = 8, blue dots) endophyte-infected forages. The red and blue dots represent linear combinations of the relative expression data, including expression values and variances, of the 26,675 gene transcripts in each Bovine GeneChip.

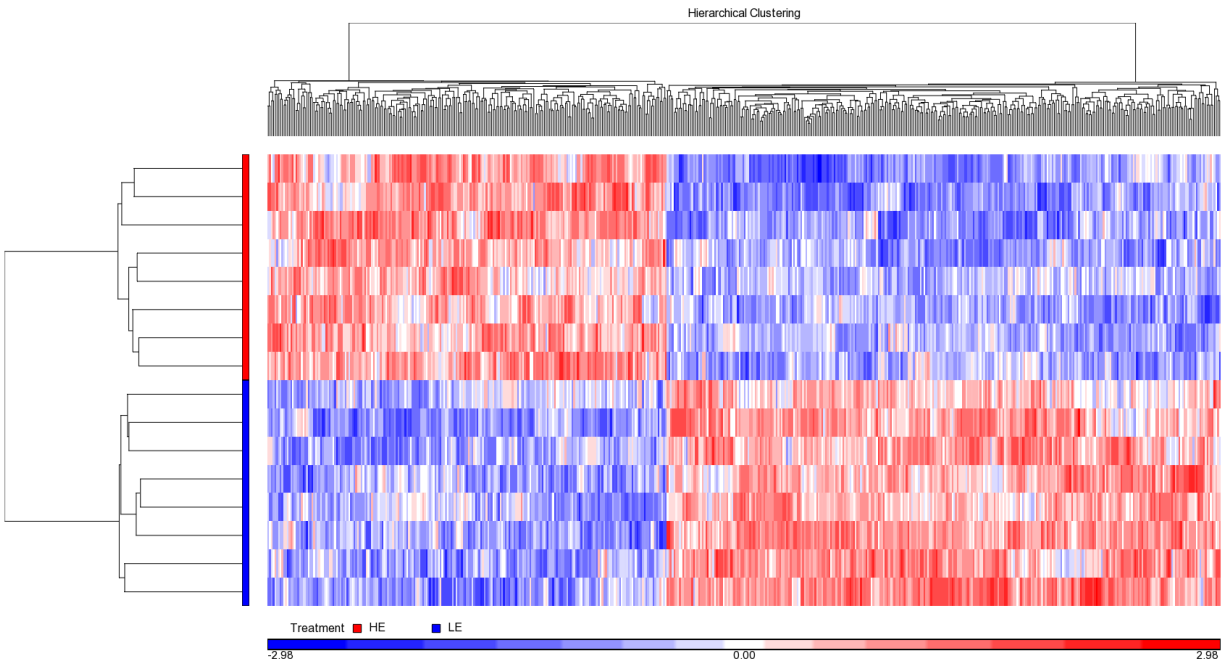

Figure 4.4. Hierarchical cluster analysis of the 542 “focus” genes selected as differentially expressed (ANOVA P-values of < 0.001 and false discovery rates of ≤ 5%) by the pituitary of steers grazing high- (HE, n = 8) vs. low- (LE, n = 8) endophyte-infected forages. As indicated by the legend color box, white color in the middle represents the mean value, 0; red color represents gene expression levels above the mean expression; and blue color denotes expression below the mean. The intensity of the color reflects the relative intensity of the fold change.

CHAPTER 5. Forms of Selenium in Vitamin-mineral Mixes Differentially Affect the Expression of Genes Responsible for Prolactin, ACTH, and α-MSH Synthesis and Mitochondrial Dysfunction in Pituitaries of Steers Grazing Endophyte-infected Tall Fescue[1]

5.1 Abstract

The goal of this study was to test the hypothesis that sodium selenite (inorganic Se, ISe), SEL-PLEX (organic forms of Se, OSe), vs. a 1:1 blend (MIX) of ISe and OSe in a basal vitamin-mineral (VM) mix would differentially alter pituitary transcriptome profiles in growing beef steers grazing an endophyte-infected tall fescue (E+) pasture. Predominately Angus steers (BW = 183 ± 34 kg) were randomly selected from fall-calving cows grazing E+ pasture and consuming VM mixes that contained 35 ppm Se as ISe, OSe, or MIX forms. Steers were weaned, depleted of Se for 98 d, and subjected to summer-long common grazing of a 10.1 ha E+ pasture containing 0.51 ppm ergot alkaloids. Steers were assigned (n = 8 per treatment) to the same Se-form treatments on which they were raised. Selenium treatments were administered by daily top-dressing 85 g of VM mix onto 0.23 kg soyhulls, using in-pasture Calan gates. As previously reported, serum prolactin was greater for MIX (52%) and OSe (59%) steers vs. ISe. Pituitaries were collected at slaughter and changes in global and selected mRNA expression patterns determined by microarray and real-time reverse transcription PCR analyses, respectively. The effects of Se treatment on relative gene expression were subjected to one-way ANOVA. The form of Se affected the expression of 542 annotated genes ($P < 0.005$). Integrated Pathway Analysis found a canonical pathway network between prolactin and pro-opiomelanocortin (POMC)/ACTH/ α-MSH synthesis-related proteins and that

mitochondrial dysfunction was a top-affected canonical pathway. Targeted reverse transcription-PCR analysis found that the relative abundance of mRNA encoding prolactin and POMC/ACTH/ α-MSH synthesis-related proteins was affected ($P < 0.05$) by the form of Se, as were ($P \leq 0.05$) mitochondrial dysfunction-related proteins (CYB5A, FURIN, GPX4, PSENEN). OSe steers appeared to have a greater prolactin synthesis capacity (more PRL mRNA) vs. ISe steers through decreased dopamine type two receptor signaling (more DRD2 mRNA), whereas MIX steers had a greater prolactin synthesis capacity (more PRL mRNA) and release potential by increasing thyrotropin-releasing hormone concentrations (less TRH receptor mRNA) than ISe steers. OSe steers also had a greater ACTH and α-MSH synthesis potential (more POMC, PCSK2, CPE, and PAM mRNA) than ISe steers. We conclude that form of Se in VM mixes altered expression of genes responsible for prolactin and POMC/ACTH/α-MSH synthesis, and mitochondrial function, in pituitaries of growing beef steers subjected to summer-long grazing an E+ pasture.

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KEYWORDS: ACTH, cattle, fescue toxicosis, mitochondria, prolactin, selenium supplementation

5.2 Introduction

Two simultaneous challenges faced by many south-eastern United States cattle producers are fescue toxicosis and Se deficiency. Fescue toxicosis results from consumption of ergot alkaloids found in *Epichloe coenophialum*-infected tall fescue (*Lolium arundinaceum*) pastures and is a clinical condition consisting of impaired metabolic, vascular, growth, and reproductive processes in cattle (Strickland et al., 2011). Reduced serum prolactin is a recognized marker of fescue toxicosis (Goetsch et al., 1987; Davenport et al., 1993). Selenium-poor soils in this same geographic region result in Se-deficient forages necessitating the need to provide supplemental Se (Dargatz and Ross, 1996). Inorganic Se (ISe, sodium selenite) is the most common form of Se supplemented in cattle diets, whereas organic forms of Se (OSe) derived from specially cultivated *Saccharomyces cerevisiae* also are available and approved for use in beef cattle diets.

Serendipitously, it was found that expression of several genes downregulated in the liver (Liao et al., 2015) and pituitary (Li et al., 2017) of steers grazing high vs. low endophyte-infected forages were upregulated in cattle by consumption of a 1:1 blend of ISe:OSe (MIX) in vitamin-mineral (VM) mixes (Matthews and Bridges, 2014; Matthews et al., 2014). Moreover, it was determined subsequently that steers subjected to summer-long grazing of endophyte-infected pasture and supplemented (3 mg/d) with MIX or OSe forms of Se had greater serum prolactin concentrations than ISe-supplemented steers (Jia et al., 2018). The first goal of the present study was to test the specific hypothesis that the amount of prolactin mRNA would be greater in the pituitary tissue of the same MIX and OSe vs. ISe steers, whereas the second goal was to test the general hypothesis that the form of supplemental Se would alter pituitary transcriptome profiles.

5.3 Materials and methods

All experimental procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee.

5.3.1 Animal model

The animal management regimen and model for steers that yielded the pituitary tissue of the present experiment have been reported (Jia et al., 2018). Briefly, twenty-four predominantly Angus beef steers (BW, 182.6 ± 33.9 kg; age, 165.5 ± 14.2 d) were randomly selected from three Se phenotypic herds (8 steers/herd), which were managed under a typical forage-based (predominately endophyte-infected tall fescue), fall-calving, cow-calf production regimen. The three Se phenotypic herds had free access to VM premixes (UK Beef IRM Cow-Calf Mineral, Burkmann Feeds, Danville, KY) containing 35 ppm of inorganic Se (ISe, sodium selenite, Prince Se Concentrate; Prince Agri Products, Inc., Quincy, IL, USA), organic Se (OSe, SEL-PLEX, Alltech Inc., Nicholasville, KY), and 1:1 mix of ISe:OSe (MIX). After depletion of Se and adaptation to consuming VM premixes from in-pasture Calan gate feeders, twenty-four steers with three Se phenotypes (n = 8) started (d 0) summer-long grazing of a 10.1-ha predominately endophyte-infected tall fescue-mixed pasture (0.51 μg/g total ergot alkaloids) (Jia et al., 2018). Three Se form treatments were administered using in-pasture Calan gate feeders to steers with the same Se phenotypes. All three Se form treatments contained a common basal VM premix that also contained 35 ppm Se as either ISe, OSe, or MIX. After the common 86-d grazing period on pastures, steers were slaughtered in the University of Kentucky Meat Laboratory (Lexington, KY) over a 26-d period.

Throughout the slaughter period, steers continued on their respective Se treatment. Details of the slaughter period and process have been reported (Jia et al., 2018).

5.3.2 Sample collection and RNA preparation

Steers were stunned by captive bolt pistol and exsanguinated. Within 10 to 12 min of death, the whole pituitary was collected from each animal, placed in a foil pack, flash-frozen in liquid nitrogen, and stored at -80°C. Four pituitary glands (two ISe, one OSe, one MIX) were not used in the microarray analysis because of tissue damage incurred during the collection process. As a result, six pituitaries for ISe and seven pituitaries for both OSe and MIX treatment groups were subjected to RNA analyses.

Total RNA was extracted from the whole frozen pituitary tissue and its purity and integrity determined as described (Li et al., 2017). Extracted RNA samples had an average concentration of 706 ng/μl and were of high purity with 260:280 nm absorbance ratios ranging from 1.85 to 2.05 and 260:230 nm absorbance ratios ranging from 2.09 to 2.50. All RNA samples had 28S:18S rRNA absorbance ratios greater than 1.8 and RNA integrity numbers greater than 8.9.

5.3.3 Microarray analysis

The GeneChip Bovine Gene 1.0 ST Array (Affymetrix, Inc., Santa Clara, CA) was used to investigate the effect of Se treatment on bovine pituitary gene expression profiles. Microarray analysis was conducted according to the manufacturer's standard protocol at the University of Kentucky Microarray Core Facility as described (Li et al., 2017), using 3 μg of RNA per sample (chip). All the GeneChip transcripts were annotated using the NetAffx annotation database for gene expression on Bovine GeneChip Array ST 1.0, provided by the manufacturer

(http://www.affymetrix.com/analysis/index.affx, last accessed in January 2018, annotation file last updated in May 2016). Quality control of the microarray hybridization and data presentation was performed by MA plot on all the gene expression values and by box plot on the control probe sets on the Affymetrix chips (data not shown). Pearson (Linear) Correlation generated the similarity matrix (last accessed in January 2018, PGS 7.17.0918). The average correlation between any pair of the 20 GeneChips was 0.96 (Supplementary Figure S1), and all GeneChips were further analyzed. Principal component analysis (**PCA**) was performed to elucidate the quality of the microarray hybridization and visualize the general data variation among the chips (Partek, 2009). To assess treatment effects (ISe vs. OSe vs. MIX) on the relative expression of the pituitary gene transcripts, qualified microarray data were subjected to one-way ANOVA using the same PGS software. To achieve a greater degree of confidence (i.e., a more conservative approach), transcripts showing treatment effects at the significance level of $P < 0.005$ (false discovery rate of ≤ 18.8%) were defined as differentially expressed. These differentially expressed genes/gene transcripts (**DEG**) were subjected to hierarchical clustering analysis using PGS software and to canonical, functional, and network pathway analyses using the Core Analysis program of Ingenuity Pathway Analysis online software (IPA, Build version 470319M, Content version 43605602; http://www.ingenuity.com [accessed in March, 2018]; Ingenuity Systems, Inc., Redwood City, CA).

All the microarray *.cel files collected by Command Console plus the GC Robust Multichip Averaging-corrected data processed by PGS software of this manuscript have been deposited in the National Center for Biotechnology Information's Gene Expression

Omnibus (**GEO;** http://www.ncbi.nlm.nih.gov/geo/) [released May 25, 2018]), are compliant with the minimum information about a microarray experiment (**MIAME**) guidelines (Brazma et al., 2001), and are accessible through GEO series accession number GSE114893.

5.3.4 Real-time reverse transcription (RT)-PCR analysis

Primer sets for genes selected for real-time RT-PCR analysis (Supplementary Table S1) were designed using the NCBI Pick Primers online program against RefSeq sequences (accessed March to November 2017), except for s-PRLR and l-PRLR, which have been reported (Thompson et al., 2011). Real-time RT-PCR was performed as described (Li et al., 2017) using 1 μg of RNA used for each reverse transcription reaction. Gene expression was analyzed by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

The resulting real-time RT-PCR products were purified using a PureLink Quick Gel Extraction Kit (Invitrogen) and sequenced at Eurofins Scientific (Eurofins, Louisville, KY). Sequences were compared with the corresponding RefSeq mRNA sequences used as the templates for primer set design. The sequences of the primers and the resulting sequence-validated real-time RT-PCR reaction amplicons for selected DEG and the endogenous control genes RPS11, TFRC, and UBC are presented in Supplementary Table S1 and Supplementary Figure S2, respectively. All sequenced amplicons had at least 98% identity with their template sequences. Three constitutively expressed genes (RPS11, TFRC, and UBC) were used and their CT values were not affected (P = 0.59, 0.51, 0.66; respectively) by Se-form treatments. Thus, the geometric mean expression of RPS11, TFRC, and UBC was used to normalize the relative

quantities of the selected DEG mRNA expression, and all RT-PCR reactions were conducted in triplicate.

5.3.5 Statistical analysis

Steers were the experimental units. To test for Se treatment effects on the relative expression of the pituitary gene transcripts, microarray hybridization data were subjected to one-way ANOVA using the PGS software as described in the "Microarray Analysis" section above. To determine the effects of treatment, the relative expression levels of selected DEG analyzed by real-time RT-PCR were subjected to one-way ANOVA using the GLM procedure of the SAS statistical software package (version 9.4; SAS Inst., Inc., Cary, NC), with the Se treatment as the fixed effect. For these data, significance was declared when $P \leq 0.05$, and a tendency to differ was declared when $0.10 > P > 0.05$. When $P < 0.10$, means were separated using Fisher's LSD procedure.

5.4 Results

5.4.1 Differentially expressed genes

Principal Component Analysis of all microarray data was performed to examine the correlation and variation among the chips, revealing a total variance of 25.53% (Supplementary Figure S3). The first principal component (PC #1, x-axis) explained a median degree of variance (10%), whereas PC #2 (y-axis) and PC #3 (z-axis) explained low degrees of variance (9.1% and 6.43%, respectively). Overall, PCA clearly demonstrated that the chips within each treatment group were clustered closely together.

Individual ANOVA was conducted to identify altered expression of RNA transcripts in the pituitary tissue across Se form treatments. At the $P < 0.01$ level and a false discovery rate of < 21.5%, 948 annotated gene transcripts were identified. To refine

this analysis, 542 genes with the criteria of a false discovery rate of less than 18.8% and $P < 0.005$ were considered to be differentially expressed (Supplementary Table S2).

Hierarchical cluster analysis of the 542 DEG revealed all steers segregated within their treatment group, except for one ISe steer, which displayed a DEG pattern similar to OSe and MIX steer groups (Supplementary Figure S4).

5.4.2 Pathways and gene network analyses

To determine the physiological significance of Se treatment-induced DEG (Supplementary Table S2), bioinformatic analysis of canonical, functional, and network pathway analyses were performed. Canonical pathway analysis (Table 1) revealed ($P < 0.005$) that the top six pathways were ephrin receptor signaling (12 genes), Th1 and Th2 activation pathway (11 genes), Th1 pathway (nine genes), breast cancer regulation by stathmin1 (11 genes), ephrin B signaling (six genes), and mitochondrial dysfunction (10 genes).

To gain insight into potentially interacting canonical pathways, canonical pathway network analysis (Figure 1) revealed one network that included four DEG (CPE, CSHL1, TGFB1, TRH) and seven other affected ($P < 0.10$) genes (DRD2, PCSK1, PCSK2, POMC, PRL, VEGFA, TRHR), all of which are related to either prolactin or POMC/ACTH/α-MSH synthesis or release.

5.4.3 Real-time RT-PCR analysis of selected mRNA

Real-time RT-PCR analysis was used to corroborate the microarray analysis-identified DEG responsible for prolactin synthesis and secretion and POMC/ACTH production in Se treatment steers (Table 2). For the prolactin receptor (PRLR), unlike the microarray analysis, the RT-PCR analysis was designed to delineate the long (l-PRLR)

and short (s-PRLR) forms. With the exception of VEGFA, the ANOVA *P*-values for Se treatment effect were consistent between the two analytical techniques. For VEGFA, microarray analysis indicated that MIX steer expression of VEGFA tended to be greater (P =0.093), whereas RT-PCR analysis found no difference (P = 0.250). With regard to fold-changes, the direction of Se treatment-induced change was the same between microarray and RT-PCR analyses while the magnitude of the determined fold-changes typically was greater by RT-PCR analysis (Table 2).

The relative expression of the nine genes that constituted the Mitochondrial Dysfunction pathway was analyzed by RT-PCR to corroborate the microarray analysis (Table 3). The trend of the numeric values of the two analyses was consistent for eight of nine evaluated genes, and statistically different for five of nine genes. Specifically, both analyses revealed that the content of CYB5A, FURIN, GPX4, and PSENEN mRNA was greater ($P \leq 0.052$), or tended (P = 0.096) to be greater (COX7A2), in OSe vs. ISe steer pituitaries. In contrast, the contents of ATP5G1, LRRK2, NDUFA2, and SDHB mRNA did not differ ($P > 0.130$), as assessed by RT-PCR analysis.

Because it has been reported that mitochondrial dysfunction is highly correlated to increased oxidative stress (Prabakaran et al., 2004; Calabrese et al., 2005; Lin and Beal, 2006), to evaluate the antioxidant response to oxidative stress, even though they were not identified as DEG by microarray analysis (Supplementary Table 2), the content of catalase (CAT) and superoxide dismutase 1 (SOD1) mRNA was evaluated by RT-PCR (Table 3). The amount of SOD1 in OSe pituitaries was greater (P = 0.018) than in ISe steers, whereas the amount of CAT mRNA did not differ (P = 0.138).

5.5 Discussion

5.5.1 Animal model

The reduction of serum prolactin by cattle consuming endophyte-infected tall fescue is a physiologic hallmark of fescue toxicosis. For example, the serum prolactin concentrations in growing beef steers subjected to summer-long grazing of high endophyte-infected tall fescue were decreased 85 to 90% relative to steers grazing low endophyte infected forage (Brown et al., 2009; Jackson et al., 2015). Unlike the well-described suppression of serum prolactin in cattle consuming ergot alkaloids, the potential effect of supplemental Se form on serum prolactin and other indicators of fescue toxicosis has not been well-characterized. For reasons (Matthews and Bridges, 2014; Matthews et al., 2014) outlined in the Introduction, we conducted a trial comparing the potential ability of the form of Se in VM mixes (35 ppm) to ameliorate some of the characteristic effects of fescue toxicosis on growing beef steers. The results (Jia et al., 2018) showed that OSe and MIX steers subjected to grazing of endophyte-infected pasture had 59% ($P < 0.03$) and 52% ($P < 0.05$) more serum prolactin than ISe steers, respectively. Using the pituitaries from the same animals, the overall goal of the present study was to determine the effect of the form of supplemental Se in VM mix on expression of pituitary targeted mRNA content transcriptome profiles to gain insight into mechanisms responsible for Se-form specific concentrations of serum prolactin.

5.5.2 The content of prolactin mRNA is greater in OSe and MIX steer pituitaries

The first goal of the present study was to test the specific hypothesis that the amount of prolactin mRNA would be greater in the pituitary tissue of the same (Jia et al., 2018) MIX and OSe vs. ISe steers. As shown in Table 2, the content of prolactin mRNA

transcripts did not differ between MIX and ISe steers according to microarray analysis, whereas RT-PCR analysis found that MIX had 100% greater content of prolactin mRNA than ISe steers. In addition, OSe steers had 18% (microarray analysis) and 250% (RT-PCR analysis) greater content of prolactin mRNA than ISe steers (Table 2). Thus, we accept the original hypothesis that the amount of prolactin mRNA would be greater in the pituitary tissue of MIX and OSe vs. ISe steers.

To gain insight into the mechanisms by which MIX and OSe steers had greater amounts of serum prolactin, the second goal of this experiment was to identify candidate molecules and signaling pathways in pituitary tissue known to be associated with prolactin synthesis (Figure 2) using microarray and RT-PCR transcript analyses (Table 2).

5.5.3 OSe form of Se supplementation had greater prolactin synthesis capacity

Dopamine is one of the most influential regulators of prolactin secretion. Activation of the dopamine type two receptor (DRD2) signaling by dopamine suppresses prolactin gene (PRL) expression via the inhibition of adenylyl cyclase and prolactin exocytosis through modification of several potassium and calcium channels (Fitzgerald and Dinan, 2008; Figure 2). Ergot alkaloids contained in endophyte-infected tall fescue resemble dopamine and trigger DRD2 signaling (Larson et al., 1999), resulting in decreased PRL transcription and serum prolactin concentrations (Strickland et al., 2011). In a previous summer-long grazing trial, the abundance of DRD2 mRNA was reduced in the pituitaries of steers that had decreased serum PRL as a result of grazing high versus low endophyte-infected tall fescue (Li et al., 2017). Consistent with this observation, consumption of endophyte-infected fescue seed reduced DRD2 mRNA and density in rat

brain (Larson et al., 1994). Moreover, DRD2 mRNA and protein levels were down-regulated under constitutive hyperdopaminergia (Fauchey et al., 2000). Hence, agonists such as dopamine and ergot alkaloids negatively regulate DRD2 mRNA expression. Because OSe steers had more serum prolactin (Jia et al., 2018) and a greater pituitary content of PRL mRNA (Table 2) than ISe steers, we expected to find a greater pituitary DRD2 mRNA content in OSe steers. As expected, OSe steers did have greater pituitary DRD2 mRNA content than did ISe steers. Thus, it is possible that the serum prolactin difference between OSe and ISe steers (OSe > ISe) was caused by differential activation of DRD2 signaling (ISe > OSe) which was derived from different dopamine/ergot alkaloids concentrations (ISe > OSe) induced by different Se forms. This possibility also is consistent with the observation that consumption of ISe (selenite), but not OSe, resulted in increased dopamine concentrations in murine striatum (Tsunoda et al., 2000), where DRD2 mRNA is down-regulated by persistent stimulation of DRD2 (Chen et al., 1993).

Pituitary transcription factor Pit-1 (encoded by POU1F1) plays a pivotal role in PRL expression by binding to specific sites of promoter elements in the PRL gene and stimulating expression of prolactin mRNA (Fox et al., 1990). However, Pit-1 mRNA was not affected by Se treatment (Table 2, Figure 2). One explanation for the finding of no difference of Pit-1 mRNA but greater pituitary PRL mRNA in OSe vs. ISe steers could be that although Pit-1 is indispensable for prolactin production, an increase in Pit-1 mRNA is not necessary to promote PRL gene expression. For example, in estrogen-treated rats, lactotroph proliferation and enhanced expression of PRL mRNA but not an increase in Pit-1 mRNA have been observed (Tsukahara et al., 1994).

During DRD2-dependent inhibition of PRL gene expression, rapid histone deacetylation of prolactin promoter occurs after activation of DRD2 signaling, followed by inhibition of ERK1/2 activity, and an unchanged association between Pit-1 and the prolactin promoter (Liu et al., 2005). Hence, a Pit-1-independent, epigenetic mechanism of DRD2 signaling also may be responsible for the difference between prolactin mRNA expression levels of OSe and ISe steers.

5.5.4 MIX form increased prolactin synthesis and release potential

As found for OSe steers, MIX steers had a greater content of pituitary PRL mRNA (Table 2) and a greater serum prolactin concentration than ISe throughout the grazing period (Jia et al., 2018). However, in contrast to OSe steers, DRD2 mRNA content in MIX steers did not differ from ISe steers (Table 2), indicating the mechanisms by which both MIX and OSe steers had greater content of prolactin mRNA and serum prolactin levels likely differed. We have examined several other genes associated with prolactin secretion, and among them is thyrotropin-releasing hormone (TRH) the principle prolactin secretagogue, which has been reported to stimulate prolactin production in both rat pituitary cells and cow pituitary tissue (Kelly et al., 1973). TRH induces prolactin mRNA levels via activation of ERK signaling pathway with synergistic increase in intracellular Ca^{2+} (White and Bancroft, 1983; Kanasaki et al., 2002) (Figure 2). TRH also was found to induce prolactin release from lactotrophs in a dose-dependent manner (Sheward et al., 1983; Lamberts and Macleod, 1990; Freeman et al., 2000). The way TRH stimulates prolactin release is via stimulation of Ca^{2+}-dependent exocytosis in lactotrophs (Sikdar et al., 1989; Christian et al., 2007). It is known that TRHR mRNA expression is negatively regulated by TRH in rat pituitary (Oron et al., 1987; Narayanan

et al., 1992). Hence, we tested mRNA expression of TRH receptors (TRHR) which have been detected in rat lactotrophs (Hinkle and Tashjian, 1973) (Figure 2). Expression of TRHR decreased in MIX vs. ISe steers according to both microarray and real-time RT-PCR analyses (Table 2). Hence, the greater serum prolactin concentrations of MIX vs. ISe steers might be due to greater TRH concentrations available in pituitaries of MIX vs. ISe steers, which may have stimulated more prolactin synthesis and release in MIX vs. ISe steers.

Transforming growth factor-β1 (TGFB1) has been shown present in lactotrophs and is capable of inhibiting prolactin release and lactotroph proliferation (Minami and Sarkar, 1997). Selenite was reported to inhibit the expression of TGFB1 induced by LPS (Pei et al., 2010). In agreement with the above study, we found that both OSe and MIX steers had more TGFB1 mRNA than ISe steers (Table 2). This finding is contradictive to the observation that serum prolactin levels were greater in OSe and MIX vs. ISe steers. One explanation to the contradiction is that the low level of TGFB1 expression limited its inhibitory potential over prolactin release, as the magnitude of TGFB1 mRNA expression appears to be lower than other genes in this network (e.g. 64-fold less than POU1F1 mRNA based on raw CT value, data not shown).

As mentioned above, we conducted RT-PCR analysis of several other genes with regard to prolactin synthesis and release, including VIP, GAL, GHRHR, VEGFA, and CSH2 based on IPA network analysis (Figure 1) and a previous study (Li et al., 2017). We also evaluated PRLR mRNA expression of both short-form and long form. However, neither microarray nor RT-PCR showed mRNA expression of these genes above affected by Se treatment.

Besides synthesis and release, metabolic clearance of prolactin may contribute to the differences in serum prolactin concentrations. That is, the kidney has been reported to metabolize two-thirds of circulating prolactin (Emmanouel et al., 1981). Hence, future studies need to examine potential Se treatment-induced differences in prolactin clearance using the same steer model.

5.5.5 OSe form of Se supplementation increased POMC/ACTH/α-MSH synthesis potential

Pro-opiomelanocortin (POMC) is a precursor polypeptide encoded by gene POMC and is synthesized mainly by corticotrophs of the anterior pituitary. Adrenocorticotropic hormone and alpha-melanocyte-stimulating hormone (α-MSH) are two important hormones derived from POMC and secreted by the anterior pituitary and intermediate lobe of the pituitary, respectively (Figure 3). Adrenocorticotropic hormone induces the adrenal cortex to secrete glucocorticoids (Schwyzer, 1977), whereas α-MSH affects feeding behavior, energy homeostasis, and inflammation (Gantz and Fong, 2003). Previous research found that the liver tissue of beef steers grazing high vs. low endophyte-infected tall fescue and consuming ad libitum amounts of ISe-containing VM mix had increased amounts of mitochondrial mass, capacity for ATP synthesis, and amino acid-derived gluconeogenesis (Brown et al., 2009; Liao et al., 2015). These processes may have been coordinated through the glucocorticoid receptor-mediated pathway (Liao et al., 2015). A subsequent gene expression study of the pituitaries from these same steers (Li et al., 2017) found that the potential for pituitary POMC/ACTH synthesis was reduced in steers consuming forage with the high amounts of endophyte-infected tall fescue. This understanding, plus the finding that selenite inhibited

glucocorticoid receptor hormone binding (Tashima et al., 1989), led to the general hypothesis of the present study that the form of supplemental Se would differentially affect mRNA content of pituitary genes related to POMC/ACTH synthesis in steers grazing endophyte-infected tall fescue.

In pituitary corticotrophs, proprotein convertase 1 (encoded by the PCSK1 gene) is expressed and cleaves POMC, producing $ACTH_{1-39}$, β-endorphin, β-lipotrophin, amino-terminal peptide, and joining peptide (Millington, 2007). That the abundance of both POMC and PCSK1 mRNA was increased in pituitaries of OSe vs. ISe steers (Table 2) indicates that OSe steers possessed a greater POMC/ACTH synthesis capacity in OSe steers. To complete the assessment of Se treatment effects on the POMC/ACTH/α-MSH synthesis pathway (Figures 1 and 3) the expression of PCSK2, CPE, and PAM was evaluated (Table 2). Collectively, the results indicate that OSe steers possess greater POMC, ACTH, and α-MSH synthesis potential than ISe steers, and a greater α-MSH synthesis potential than MIX steers. As for prolactin, the exact physiological consequences of Se form-altered expression of ACTH and α-MSH remains to be determined.

5.5.6 Functional analysis of the genes involved in mitochondrial dysfunction and antioxidant defense

As noted above, gene expression profiling indicated that the liver of steers grazing high vs. low endophyte-infected tall fescue and consuming ISe as a Se source had increased mitochondrial mass and respiratory chain mediated ATP synthetic capacity (Liao et al., 2015). The role that Se plays in preserving mitochondrial function is controversial. Whereas Se induces apoptosis associated with ROS accumulation and

mitochondrial dysfunction (Guan et al., 2009), and selenite is detrimental to mitochondrial membrane potential by induction of mitochondrial permeability transition through thiol-oxidation (Kim et al., 2002), Se also is known to attenuate apoptosis (of at least damaged spinal cord tissue) through protection of mitochondrial function (Yeo et al., 2008) and shows a protective effect on cadmium-induced apoptosis in mice kidney (Wang et al., 2013). Because canonical pathway analysis of pituitary DEG identified "mitochondrial dysfunction" as one of the top pathways affected by Se treatment (Table 1), expression of DEG involved in mitochondrial dysfunction pathways was further examined by RT-PCR analysis, along with two genes (SOD1 and CAT) encoding key antioxidant enzymes.

Although microarray analysis showed that OSe steers expressed more NDUFA2, COX7A2, SDHB, and ATP5G1 mRNA content than ISe steers, RT-PCR analysis (Table 3) corroborated increased expression of NDUFA2 and COX7A2. Collectively, these data indicate that ISe had a reduced electron transport chain capacity than OSe steers, thereby likely less ATP generation and more damaging ROS in mitochondria (Bosetti et al., 2002; Musatov and Robinson, 2012; Saito et al., 2016;). In terms of mitigating oxidative stress, genes involved with control of ROS production (LRRK2, CYB5A, PSNENEN) and antioxidant production and use (FURIN, CAT, SOD1, GPx4) were evaluated. OSe pituitaries expressed greater levels of SOD1, GPx4, and PSENEN than ISe steers and CYB5A and FURIN than ISe and MIX steers. Collectively, these findings strongly indicate that the pituitaries of OSe steers had a greater capacity to manage oxidative stress vs. ISe steers (Mates et al., 1999; Zangar et al., 2004; Heo et al., 2010).

In summary, consumption of 3 mg Se/d in VM mixes as OSe, MIX, or ISe differentially affected the expression of genes responsible for the synthesis or release of prolactin and POMC/ACTH/α-MSH, and for mitochondrial function, in the pituitaries of growing beef steers grazing an endophyte-infected tall fescue pasture. Consumption of OSe resulted in a greater prolactin synthesis capacity, whereas consumption of MIX resulted in increased prolactin synthesis and release potential, both of which resulted in greater serum prolactin concentrations in OSe and MIX steers vs. ISe steers, respectively. In addition, consumption of OSe resulted in greater POMC/ACTH/α-MSH synthesis potential than consumption of ISe and MIX forms of Se, and a better capacity to manage against mitochondrial dysfunction and oxidative stress, than consumption of ISe. The implications from these findings are that the inclusion of an organic form of Se in free-choice vitamin-mineral mixes can partially ameliorate the negative impact of fescue toxicosis on growing beef steers by restoration of both prolactin and POMC/ACTH synthesis capacities. In addition, because the role of prolactin is best understood in regulating lactation (Lamberts and Macleod, 1990; Freeman et al., 2000), it may be of especial commercial importance to evaluate the potential effect of MIX and OSe forms of Se in VM mixes to ameliorate the negative effects of grazing endophyte-infected tall fescue in lactating/sucking cow/calf pairs.

Table 5.1. Top six IPA-identified canonical pathways of genes differentially expressed by pituitary tissue of steers grazing endophyte-infected tall fescue and supplemented with 3 mg Se/d in vitamin-mineral mixes as either sodium selenite (ISe), SEL-PLEX (OSe), or a 1:1 mix of ISe and OSe (MIX).

Canonical Pathway	Number [1]	Gene Symbol	Ratio[2]	-log (*P*-value)
Ephrin Receptor Signaling	12	*ROCK2,EPHB6,ITGA3,SDC2,RACK1,LIMK2,EFNB3,STAT3,RAP1A,EPHA2,GRINA,LIMK1*	0.068	3.57
Th1 and Th2 Activation Pathway	11	*PSENEN,TGFB1,IL1RL1,LTA,IL6R,mir-155,VAV1,IL27RA,STAT3,IFNAR1,IL18R1*	0.060	2.83
Th1 Pathway	9	*PSENEN,LTA,IL6R,mir-155,VAV1,IL27RA,STAT3,IFNAR1,IL18R1*	0.066	2.74
Breast Cancer Regulation by Stathmin1	11	*ROCK2,TUBB4B,PPP2R3A,PPP1R14D,PRKCD,RACK1,ARHGEF1,LIMK2,ARHGEF3,PPP1CA,LIMK1*	0.053	2.43
Ephrin B Signaling	6	*ROCK2,EPHB6,RACK1,VAV1,EFNB3,LIMK1*	0.080	2.38
Mitochondrial Dysfunction	10	*FURIN,SDHB,ATP5G1,PSENEN,COX7A2,LRRK2,CYB5A,GPX4,NDUFAB1,NDUFA2*	0.053	2.29

[1]The number of genes (listed in the "Symbol" column) associated with the particular canonical pathway.

[2]The ratio is calculated as the number of genes in a given pathway that meet cutoff criteria (e.g., the ANOVA *P*-value for the differential expression among Se groups is less than 0.005) divided by the total number of genes that make up that pathway.

Table 5.2. Comparison of microarray- and real-time RT-PCR (RT-PCR)-determined relative expression of prolactin and POMC/ACTH synthesis related genes in pituitary tissue of steers grazing endophyte-infected tall fescue and supplemented with 3 mg Se/d in vitamin-mineral mixes as either sodium selenite (ISe), SEL-PLEX (OSe), or a 1:1 mix of ISe and OSe (MIX).

Gene	Gene Name	Microarray[1]				RT-PCR[2]			
		Treatment[3,4]			*P*-	Treatment[3,4]			*P*-
		ISe	MIX	OSe	value	ISe	MIX	OSe	value
Item									
Prolactin Synthesis Related Genes									
DRD2	Dopamine receptor D2	1.00[a]	1.15[ab]	1.27[b]	0.027	1.13[a]	1.64[ab]	2.48[b]	0.039
POU1F1	POU class 1 homeobox 1	1.00	1.06	1.05	0.530	1.02	1.11	0.87	0.277
PRL	Prolactin	1.00[a]	1.12[ab]	1.18[b]	0.022	1.02[a]	2.08[b]	3.67[b]	0.007
TRHR	Thyrotropin releasing hormone receptor	1.00[a]	0.76[b]	0.83[ab]	0.045	1.00[a]	0.71[b]	0.81[ab]	0.039
VIP	Vasoactive intestinal peptide	1.00	1.64	1.43	0.331	1.21	1.74	2.31	0.311
GAL	Galanin/GMAP prepropeptide	1.00	1.03	1.00	0.821	1.32	2.10	2.35	0.182
VEGFA	Vascular endothelial growth factor A	1.00[a]	1.15[b]	1.05[ab]	0.093	1.01	1.18	1.03	0.250
TGFB1	Transforming growth factor beta 1	1.00[a]	1.46[b]	1.30[b]	0.001	1.02[a]	1.78[b]	2.06[c]	0.005
GHRHR	Growth Hormone Releasing Hormone Receptor	1.00	0.93	1.00	0.532	1.01	1.07	1.29	0.166
CSH2	Chorionic Somatomam	1.00	1.09	0.97	0.173	1.10	0.74	0.87	0.279

Table 5.2 (continued)

	motropin Hormone 2								
PRLR	Prolactin receptor	1.00	1.13	1.23	0.141	NA	NA	NA	NA
L-PRLR	Prolactin receptor long isoform	NA	NA	NA	NA	1.01	0.95	1.17	0.376
S-PRLR	Prolactin receptor short isoform	NA	NA	NA	NA	1.01	1.01	1.09	0.761
POMC/ACTH/α-MSH Synthesis Related Gene									
POMC	Proopiomelanocortin	1.00^{a}	1.10^{ab}	1.23^{b}	0.045	1.14^{a}	1.91^{a}	4.06^{b}	0.002
PCSK1	Proprotein convertase subtilisin/kexin type 1	1.00^{a}	1.09^{ab}	1.44^{b}	0.059	1.03^{a}	1.48^{ab}	1.68^{b}	0.076
PCSK2	Proprotein convertase subtilisin/kexin type 2	1.00^{a}	1.13^{ab}	1.33^{b}	0.074	1.03^{a}	1.14^{ab}	1.41^{b}	0.048
CPE	Carboxypeptidase E	1.00^{a}	0.98^{a}	1.04^{b}	0.002	1.01^{a}	1.01^{a}	1.31^{b}	0.003
PAM	Peptidylglycine alpha-amidating monooxygenase	1.00^{a}	1.04^{a}	1.29^{b}	0.044	1.06^{a}	1.28^{a}	1.96^{b}	0.008

[1]The abundance of gene transcripts are reported relative to the mean expression of the ISe treatment group and are expressed as fold-change of the untransformed intensity value.

[2]The abundance of gene transcripts are reported relative to the geometric mean expression of the reference genes.

[3]Values are least squares means (n = 6 for ISe, n = 7 for OSe and MIX)

[4]Means within a row that lack a common letter differ ($P < 0.05$).

Table 5.3. Comparison of microarray and real-time RT-PCR (RT-PCR) identification of mitochondrial dysfunction related genes by pituitary tissue of steers grazing endophyte-infected tall fescue and supplemented with 3 mg Se/d in vitamin-mineral mixes as either sodium selenite (ISe), SEL-PLEX (OSe), or a 1:1 mix of ISe and OSe (MIX).

Gene	Gene Name	Microarray[1]				RT-PCR[2]			
		Treatment[3,4]			*P*-value	Treatment[3,4]			*P*-value
		ISe	MIX	OSe		ISe	MIX	OSe	
Item									
Mitochondrial Dysfunction Related Genes									
ATP5G1	ATP Synthase, H+ Transporting, Mitochondrial Fo Complex Subunit C1 (Subunit 9)	1.00[a]	1.22[b]	1.26[b]	0.004	1.04	1.24	1.31	0.266
COX7A2	Cytochrome C Oxidase Subunit 7A2	1.00[a]	1.05[a]	1.24[b]	0.001	1.00[a]	1.20[ab]	1.29[b]	0.096
CYB5A	Cytochrome B5 Type A	1.00[a]	1.00[a]	1.13[b]	0.002	1.02[a]	1.02[a]	1.27[b]	0.024
FURIN	Paired Basic Amino Acid Cleaving Enzyme	1.00[a]	1.04[a]	1.12[b]	0.002	1.04[a]	1.33[a]	1.81[b]	0.011
GPX4	Glutathione peroxidase 4	1.00[a]	1.21[b]	1.23[b]	0.004	1.03[a]	1.32[ab]	1.56[b]	0.052
LRRK2	Leucine Rich Repeat Kinase 2	1.00[a]	0.88[b]	0.79[c]	0.001	1.00	0.88	0.85	0.130
NDUFA2	NADH:Ubiquinone Oxidoreductase Subunit A2	1.00[a]	1.18[b]	1.16[b]	0.003	1.01	1.16	1.23	0.142
PSENEN	Presenilin Enhancer Gamma-Secretase Subunit	1.00[a]	1.13[b]	1.24[c]	0.001	1.01[a]	1.17[ab]	1.32[b]	0.032
SDHB	Succinate Dehydrogenase Complex Iron Sulfur Subunit B	1.00[a]	1.03[a]	1.12[b]	0.003	1.23	1.71	1.46	0.573
Antioxidant Enzyme-encoding Genes									
CAT	Catalase	1.00	1.00	1.04	0.127	1.02	1.05	1.24	0.138

Table 5.3 (continued)

SOD1	Superoxide Dismutase 1	1.00	1.06	1.04	0.115	1.05[a]	1.35[b]	1.80[b]	0.018

[1]The abundance of gene transcripts are reported relative to the mean expression of the ISe treatment group and are expressed as fold-change of the untransformed intensity value.

[2]The abundance of gene transcripts are reported relative to the geometric mean expression of the reference genes.

[3]Values are least squares means (n = 6 for ISe, n = 7 for OSe and MIX)

[4]Means within a row that lack a common letter differ ($P < 0.05$).

Table 5.4. Primer sets used for quantitative real-time RT-PCR analysis of the selected differentially expressed genes and reference genes.

Gene	Gene Name	Template Accession number[1]	Oligonucleotide Primer Design (5' to 3' direction)	Amplicon length (bp)	Product identity (%)[2]
RPS11	Ribosomal protein S11	NM_001024568.2	F: AAGATGGCGGACATTCAGAC R: GCCCTCGAATGGAGACATTA	214	99%
TFRC	Transferrin Receptor	NM_001206577.1	F: CCAGGTTTAGTCTGGCTCGG R: GGTCTGCCCAGAATATGCGA	339	99%
UBC	Ubiquitin C	NM_001206307.1	F: TAGGGGTGGGTTAGAGTTCAAG R: ACCACCTCCCTGCTGGTATT	258	100%
DRD2	Dopamine receptor D2	NM_174043.2	F: CGACCTTTCTCTGGGGCTTT R: TTGGGCTTCTGCTTCTCTGG	234	100%
POU1F1	POU class 1 homeobox 1	NM_174579	F: AGCTGTGCATGGCTCTGAAT R: AGGCTTGTCTTCACCCGTTT	354	100%

Table 5.4 (continued)

PRL	Prolactin	NM_173953.2	F: AGAACAAGCCCAACAGACCC R: AGTCCTGACCACACAGGGTA	252	99%
TRHR	Thyrotropin releasing hormone receptor	M_174203.1	F: GCGATCTGTCACCCCATCAA R: ATCCGTAGAGGACAGTGGCT	262	100%
VIP	Vasoactive intestinal peptide	NM_173970.3	F: CTGGTTCAGCTGTAAGGGCA R: TCAGCCAGCGCATCTTGTAA	325	100%
GAL	Galanin/GMAP prepropeptide	NM_173914.2	F: CACCGGTGAAGGAGAAGAGAG R: GGCGTCTTTGAGATGCAGGAA	230	100%
VEGFA	Vascular endothelial growth factor A	NM_174216.2	F: GCAAGAAAATCCCTGTGGGC R: CGTCTGGTTCCCGAAACCCT	210	100%
TGFB1	Transforming growth factor beta 1	NM_001166068.1	F: GCGGCCAGATTTTGTCCAAG R: GCTGTGCGAGCTAGACTTCA	242	98%

Table 5.4 (continued)

GHRHR	Growth Hormone Releasing Hormone Receptor	NM_181020.3	F: CTGTAACAGTCCTGTGTAAGGT R: GTCTTCGAAGGCCAACTTGC	218	100%
CSH2	Chorionic Somatomammotropin Hormone 2	NM_181007.2	F: CCCCATGCTAAGCCCACAAT R:TTTCTCTCAGAGGTAGGGATGGA	243	100%
L-PRLR	Prolactin receptor long isoform	NM_001039726.2	F: GCCATCCTTTCTGCTGTCAT R: CCCTTCTCCAGCAGATGAAC	136	100%
S-PRLR	Prolactin receptor short isoform	NM_174155.3	F: GCCATCCTTTCTGCTGTCAT R: AAGGCGAGAAGGCTGTGATA	151	99%
POMC	Proopiomelanocortin	NM_174151.1	F: AGCTTCCCCGTGACAGAGC R: CTGCTACCATTCCGACGGC	317	99%
PCSK1	Proprotein convertase subtilisin/kexin type 1	NM_174412.2	F: TGATCGTGTGATATGGGCGG R: GGCCTCCGGATCATAGTTGG	277	99%

Table 5.4 (continued)

PCSK2	Proprotein convertase subtilisin/kexin type 2	NM_174413.3	F: TGGTTTTTGCATCTGCTGAG R: CCTCTTTACCCTGGGGTCTC	233	97%
CPE	Carboxypeptidase E	NM_173903.4	F: GCTTAGCTCGGGCATACTCA R: CACAGAGAGGGTGGCGTTAG	347	99%
PAM	Peptidylglycine alpha-amidating monooxygenase	NM_173948.2	F: ACCAACACCGTGTGGAAGTT R: GTTCAGAATCTCCAAAGGCCC	301	99%
ATP5G1	ATP Synthase, H+ Transporting, Mitochondrial Fo Complex Subunit C1 (Subunit 9)	NM_176649.3	F: CACCGGGGCACTACTCATTT R: AAGAGCCCCATAGCCTCAGA	363	99%
COX7A2	Cytochrome C Oxidase Subunit 7A2	NM_175807.1	F: ACTGAGCCAAGATGCTACGG R: ACCCAACTGATTGCTGGGAG	295	99%

Table 5.4 (continued)

CYB5A	Cytochrome B5 Type A	NM_174033.3	F: AAGACTGCTTTGGTCCAGGG R: GACAGCTCAGCATGGTGGTA	263	99%
FURIN	Paired Basic Amino Acid Cleaving Enzyme	NM_174136	F: GCATCGAGAAGAACCACCCA R: CTCCACGGCATCTGTCACTT	245	100%
GPX4	Glutathione peroxidase 4	NM_174770.3	F: GATCAAAGAGTTCGCCGCTG R: CCATACCGCTTCACCACACA	198	100%
LRRK2	Leucine Rich Repeat Kinase 2	NM_001206086.2	F: AGGAATCCGATGCTTTGGCA R: ACTGCAATGCTGGGTCTTGA	300	99%
NDUFA2	Oxidoreductase Subunit A2	NM_175815.2	F: CGTATCCATTTGTGCCAGCG R: ACGTTCTCCAGGGCTCTAGT	224	100%
PSENEN	Presenilin Enhancer Gamma-Secretase Subunit	NM_001008669.1	F: GCGTGGTTGTTTGTGATCCT R: TTGATTTGGCTCTGCTCCGT	251	100%

Table 5.4 (continued)

SDHB	Succinate Dehydrogenase Complex Iron Sulfur Subunit B	NM_001040483.1	F: AGAGACGACTTCACGGAGGA R: CTGAAGGAACTCAGGGGTGA	238	99%
CAT	Catalase	NM_001035386.2	F: CTATCCTGACACTCACCGCC R: GAAAGTCCGCACCTGAGTGA	268	99%
SOD1	Superoxide Dismutase 1	NM_174615.2	F: TTGGAGACCTGGGCAATGTG R: TTACACCACAGGCCAAACGG	204	99%

[1]The contents in the parentheses associated with each gene symbol are the accession numbers of the sequences retrieved from the NCBI RefSeq database and used as templates for designing primers and probes.

[2]All the real-time RT-PCR products were validated by sequencing. The identity values (%) presented are the base-pair ratios between the number of identical base pairs and the total amplicon length.

Table 5.5. DEG list ($P < 0.005$, 542 annotated genes), expressed by pituitaries collected from steers grazing endophyte-infected tall fescue and supplemented with 3 mg Se/d in vitamin-mineral mixes as either sodium selenite (ISe), SEL-PLEX (OSe), or a 1:1 mix of ISe and OSe (MIX).

Dietary Se treatment						
Transcript ID	Gene Symbol	Gene Description	ISe	OSe	MIX	SEM *P*-value
12815073	ABCC6	ATP-binding cassette, sub-family C (CFTR	1.00[a]	-1.10[b]	-1.19[c]	0.0240.003072
12812150	ABHD11	abhydrolase domain containing 11	1.00[a]	1.31[b]	1.20[c]	0.0360.000158
12763396	ABR	active BCR-related gene	1.00[a]	1.12[b]	1.09[b]	0.0230.002440
12681108	ACAD11	acyl-CoA dehydrogenase family, member 11	1.00[a]	-1.13[b]	-1.09[b]	0.0210.000701
12843399	ACADM	acyl-CoA dehydrogenase, C-4 to C-12 straight chain	1.00[a]	-1.13[b]	-1.03[a]	0.0210.001467
12714006	ACOT8	acyl-CoA thioesterase 8	1.00[a]	1.19[b]	1.08[c]	0.0320.003085
12860803	ACSS3	acyl-CoA synthetase short-chain family member 3	1.00[a]	-1.17[b]	-1.09[c]	0.0270.003610
12811304	ACTB	actin, beta	1.00[a]	1.06[b]	1.13[c]	0.0210.002141
12703348	ACTR1B	ARP1 actin-related protein 1 homolog B, centractin beta (yeas	1.00[a]	1.12[b]	1.04[a]	0.0180.000423
12700297	ADAM17	ADAM metallopeptidase domain 17	1.00[a]	-1.20[b]	-1.11[c]	0.0290.003166
12819148	ADD3	adducin 3 (gamma)	1.00[a]	-1.19[b]	-1.13[b]	0.0260.000332
12714706	ADIG	adipogenin	1.00[a]	-1.00[a]	-1.16[b]	0.0290.004786
12823127	AFAP1L2	actin filament associated protein 1-like 2	1.00[a]	-1.05[a]	-1.14[b]	0.0150.001903
12816222	AGFG2	ArfGAP with FG repeats 2	1.00[a]	1.10[b]	1.03[a]	0.0180.003134

Table 5.5 (continued)

12785796	AGXT2	alanine--glyoxylate aminotransferase 2	1.00[a]	1.34[b]	-1.00[a]	0.0520.003621
12701187	AHSA2	AHA1, activator of heat shock 90kDa protein ATPase homolog 2 (	1.00[a]	-1.06[a]	1.22[b]	0.0280.004122
12839104	AMIGO1	adhesion molecule with Ig-like domain 1	1.00[a]	1.11[b]	-1.06[a]	0.0330.003137
12681779	AMOTL2	angiomotin like 2	1.00[a]	1.18[b]	-1.03[a]	0.0350.001440
12770437	AMZ2	archaelysin family metallopeptidase 2	1.00[a]	1.21[b]	1.15[b]	0.0310.000537
12758448	ANKRD11	ankyrin repeat domain 11	1.00[a]	-1.12[b]	1.06[a]	0.0310.003210
12693457	APEX1	APEX nuclease (multifunctional DNA repair enzyme) 1	1.00[a]	1.19[b]	1.16[b]	0.0330.004432
12729444	API5	apoptosis inhibitor 5	1.00[a]	-1.09[b]	1.04[a]	0.0210.002289
12699389	APLF	aprataxin and PNKP like factor	1.00[a]	-1.25[b]	-1.17[b]	0.0330.001334
12842977	APOA1BP	apolipoprotein A-I binding protein	1.00[a]	1.14[b]	1.19[b]	0.0280.001339
12757598	APRT	adenine phosphoribosyltransferase	1.00[a]	1.20[b]	1.09[c]	0.0330.002713
12696328	AQR	aquarius homolog (mouse)	1.00[a]	-1.07[b]	1.01[a]	0.0160.004085
12712642	ARGLU1	arginine and glutamate rich 1	1.00[a]	-1.13[b]	-1.08[c]	0.0130.000477
12731455	ARHGAP42	Rho GTPase activating protein 42	1.00[a]	-1.12[b]	1.02[a]	0.0230.002245
12757115	ARHGEF1	Rho guanine nucleotide exchange factor (GEF) 1	1.00[a]	1.15[b]	1.12[b]	0.0260.004440
12797402	ARHGEF3	Rho guanine nucleotide exchange factor (GEF) 3	1.00[a]	-1.25[b]	-1.18[b]	0.0250.000387
12878680	ARID3A	AT rich interactive domain 3A (BRIGHT-like)	1.00[a]	1.19[b]	1.34[c]	0.0420.002240

Table 5.5 (continued)

12680069	ARL13B	ADP-ribosylation factor-like 13B	1.00[a]	-1.15[b]	-1.07[c]	0.0240.001274
12818542	ARL6IP1	ADP-ribosylation factor-like 6 interacting protein 1	1.00[a]	-1.18[b]	-1.05[a]	0.0250.000310
12798781	ARL6IP5	ADP-ribosylation-like factor 6 interacting protein 5	1.00[a]	1.09[b]	1.00[a]	0.0160.001479
12872066	ARL9	ADP-ribosylation factor-like 9	1.00[a]	1.33[b]	1.44[b]	0.0690.004465
12730790	ARRB1	arrestin, beta 1	1.00[a]	1.18[b]	1.09[c]	0.0320.003087
12885849	ARRDC2	arrestin domain containing 2	1.00[a]	-1.31[b]	-1.15[c]	0.0510.002569
12793470	ARRDC4	arrestin domain containing 4	1.00[a]	-1.31[b]	-1.17[c]	0.0410.000442
12857545	ART4	ADP-ribosyltransferase 4 (Dombrock blood group)	1.00[a]	-1.26[b]	-1.36[b]	0.0560.003082
12861962	ASCL1	achaete-scute complex homolog 1 (Drosophila)	1.00[a]	1.38[b]	1.25[b]	0.0510.000848
12854099	ASNS	asparagine synthetase (glutamine-hydrolyzing)	1.00[a]	1.21[b]	1.15[c]	0.0210.000033
12810391	ASXL3	additional sex combs like 3 (Drosophila)	1.00[a]	1.03[a]	1.18[b]	0.0220.000595
12871836	ATP10D	ATPase, class V, type 10D	1.00[a]	1.28[b]	1.22[b]	0.0420.001896
12909023	ATP11C	ATPase, class VI, type 11C	1.00[a]	-1.24[b]	-1.06[a]	0.0420.003799
12880589	ATP13A1	ATPase type 13A1	1.00[a]	1.15[b]	1.18[b]	0.0310.002721
12749501	ATP1A3	ATPase, Na+	1.00[a]	1.15[b]	1.07[c]	0.0250.002322
12767689	ATP5G1	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit	1.00[a]	1.26[b]	1.22[b]	0.0510.003783
12761235	ATP6V0A1	ATPase, H+ transporting, lysosomal V0 subunit a1	1.00[a]	1.09[b]	1.02[a]	0.0170.003025
12848186	ATP8B2	ATPase, class I, type 8B, member 2	1.00[a]	1.22[b]	1.18[b]	0.0340.000823

Table 5.5 (continued)

12862844	ATXN7L3B	ataxin 7-like 3B	1.00[a]	1.11[b]	1.10[b]	0.0240.003916
12700080	B3GNT2	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 2	1.00[a]	-1.33[b]	-1.21[b]	0.0510.001367
12892724	B4GALT1	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide	1.00[a]	1.15[b]	1.19[b]	0.0300.001027
12805572	BAK1	BCL2-antagonist	1.00[a]	1.13[b]	1.15[b]	0.0280.003007
12751423	BANP	BTG3 associated nuclear protein	1.00[a]	-1.34[b]	-1.01[a]	0.0500.001295
12801533	BAT3	HLA-B associated transcript 3	1.00[a]	1.18[b]	1.16[b]	0.0370.004401
12858722	BAZ2A	bromodomain adjacent to zinc finger domain, 2A	1.00[a]	1.03[a]	1.21[b]	0.0210.000476
12718315	BCAS1	breast carcinoma amplified sequence 1	1.00[a]	-1.16[b]	-1.22[b]	0.0340.002844
12837242	BCAS2	breast carcinoma amplified sequence 2	1.00[a]	-1.03[a]	1.05[b]	0.0180.004750
12790583	BDKRB1	bradykinin receptor B1	1.00[a]	-1.35[b]	-1.14[c]	0.0430.002912
12798575	BHLHE40	basic helix-loop-helix family, member e40	1.00[a]	1.52[b]	1.43[b]	0.0430.000044
12801465	BMP5	bone morphogenetic protein 5	1.00[a]	-1.31[b]	-1.38[b]	0.0670.004115
12873825	BOD1L	biorientation of chromosomes in cell division 1-like	1.00[a]	-1.12[b]	1.01[a]	0.0200.000800
12813925	BOLA2B	bolA homolog 2B (E. coli)	1.00[a]	1.19[b]	1.14[b]	0.0290.003782
12754924	BOSTAUV1R430	vomeronasal 1 receptor bosTauV1R430	1.00[a]	1.34[b]	1.04[a]	0.0420.003031
12754921	BOSTAUV1R431	vomeronasal 1 receptor bosTauV1R431	1.00[a]	1.44[b]	1.02[a]	0.0580.001756

Table 5.5 (continued)

12714979	BTBD3	BTB (POZ) domain containing 3	1.00[a]	-1.22[b]	-1.13[b]	0.0320.001981
12694170	C10H14orf37	chromosome 14 open reading frame 37 ortholog	1.00[a]	1.02[a]	-1.15[b]	0.0200.001281
12698893	C11H2orf56	protein midA homolog, mitochondrial	1.00[a]	-1.15[b]	-1.12[b]	0.0260.001585
12741467	C16H1orf156	chromosome 1 open reading frame 156 ortholog	1.00[a]	-1.12[b]	1.01[a]	0.0280.004246
12740403	C16H1ORF55	chromosome 1 open reading frame 55 ortholog	1.00[a]	-1.19[b]	-1.02[a]	0.0250.000270
12828575	C1orf124	zinc finger RAD18 domain-containing protein C1orf124 homolo	1.00[a]	-1.15[b]	-1.02[a]	0.0180.000467
12806978	C23H6orf130	chromosome 6 open reading frame 130 ortholog	1.00[a]	-1.17[b]	-1.09[c]	0.0280.003790
12781409	C2H1orf144	chromosome 1 open reading frame 144 ortholog	1.00[a]	1.13[b]	1.17[b]	0.0270.001946
12776233	CAB39	calcium binding protein 39	1.00[a]	-1.13[b]	-1.02[a]	0.0220.000792
12846417	CAPZA1	capping protein (actin filament) muscle Z-line, alpha 1	1.00[a]	-1.04[a]	1.08[b]	0.0200.001444
12753658	CBFB	core-binding factor, beta subunit	1.00[a]	-1.16[b]	1.04[a]	0.0350.000799
12868256	CCDC53	coiled-coil domain containing 53	1.00[a]	-1.23[b]	-1.15[b]	0.0360.002356
12860511	CCDC77	coiled-coil domain containing 77	1.00[a]	-1.21[b]	-1.17[b]	0.0270.004501
12767023	CCL2	chemokine (C-C motif) ligand 2	1.00[a]	1.88[b]	1.73[b]	0.0980.000670
12774476	CCL20	chemokine (C-C motif) ligand 20	1.00[a]	-1.08[a]	-1.34[b]	0.0450.000806
12870891	CCNG2	cyclin G2	1.00[a]	-1.24[b]	-1.21[b]	0.0230.000890

Table 5.5 (continued)

12857478	CD63	CD63 molecule	1.00[a]	1.16[b]	1.15[b]	0.0250.000494
12841117	CDC42SE1	CDC42 small effector 1	1.00[a]	1.14[b]	1.28[c]	0.0490.003235
12808806	CDH19	cadherin 19, type 2	1.00[a]	-2.34[b]	-1.49[c]	0.1420.002230
12683809	CDV3	CDV3 homolog (mouse)	1.00[a]	-1.01[a]	1.09[b]	0.0220.002642
12884966	CDX1	caudal type homeobox 1	1.00[a]	1.12[b]	-1.08[a]	0.0380.004126
12698371	CEL	carboxyl ester lipase (bile salt-stimulated lipase)	1.00[a]	1.12[b]	-1.10[c]	0.0380.002547
12741130	CENPL	centromere protein L	1.00[a]	-1.12[b]	1.07[a]	0.0370.004458
12690629	CGRRF1	cell growth regulator with ring finger domain 1	1.00[a]	-1.08[b]	1.11[c]	0.0270.001127
12723011	CHD7	chromodomain helicase DNA binding protein 7	1.00[a]	-1.02[a]	1.12[b]	0.0200.002943
12791729	CHRNA7	cholinergic receptor, nicotinic, alpha 7	1.00[a]	1.25[b]	1.07[a]	0.0390.002142
12705319	CHST10	carbohydrate sulfotransferase 10	1.00[a]	1.16[b]	1.17[b]	0.0340.002818
12681827	CLRN1	clarin 1	1.00[a]	1.32[b]	1.18[c]	0.0380.000486
12694413	CMTM5	CKLF-like MARVEL transmembrane domain containing 5	1.00[a]	-1.04[a]	-1.20[b]	0.0310.001544
12758174	CNOT3	CCR4-NOT transcription complex, subunit 3	1.00[a]	1.16[b]	1.13[b]	0.0310.003299
12858172	CNPY2	canopy 2 homolog (zebrafish)	1.00[a]	1.23[b]	1.22[b]	0.0440.004584
12740915	CNST	consortin, connexin sorting protein	1.00[a]	-1.23[b]	1.00[a]	0.0380.000705
12895030	CNTFR	ciliary neurotrophic factor receptor	1.00[a]	1.22[b]	1.14[b]	0.0360.004783
12894487	CNTLN	centlein, centrosomal protein	1.00[a]	-1.25[b]	-1.11[c]	0.0390.003100
12908781	COL4A6	collagen, type IV, alpha 6	1.00[a]	-1.24[b]	-1.19[b]	0.0440.004031

Table 5.5 (continued)

12864037	COPZ1	coatomer protein complex, subunit zeta 1	1.00[a]	1.16[b]	1.13[b]	0.0270.002766
12867761	COQ10A	coenzyme Q10 homolog A (S. cerevisiae)	1.00[a]	1.19[b]	1.20[b]	0.0350.001360
12742723	CORO1C	coronin, actin binding protein, 1C	1.00[a]	-1.23[b]	-1.06[a]	0.0260.000245
12756789	COX4NB	COX4 neighbor	1.00[a]	1.19[b]	1.26[b]	0.0310.000128
12899530	COX7A2	cytochrome c oxidase subunit VIIa polypeptide 2 (liver)	1.00[a]	1.24[b]	1.05[a]	0.0310.000448
12741898	CPE	carboxypeptidase E	1.00[a]	1.04[b]	-1.02[a]	0.0120.002100
12678420	CPNE4	copine IV	1.00[a]	-1.23[b]	-1.25[b]	0.0480.002398
12798341	CRELD1	cysteine-rich with EGF-like domains 1	1.00[a]	1.19[b]	1.28[b]	0.0500.003031
12780237	CRYGB	crystallin, gamma B	1.00[a]	1.30[b]	1.14[c]	0.0440.004510
12807461	CYB5A	CYB5 protein	1.00[a]	1.13[b]	1.00[a]	0.0220.001616
12755641	CYBA	cytochrome b-245, alpha polypeptide	1.00[a]	1.29[b]	1.05[a]	0.0450.001481
12696721	DCP2	DCP2 decapping enzyme homolog (S. cerevisiae)	1.00[a]	-1.14[b]	1.01[a]	0.0240.000526
12860523	DCTN2	dynactin 2 (p50)	1.00[a]	1.13[b]	1.06[c]	0.0240.003064
12826569	DDIT4	DNA-damage-inducible transcript 4	1.00[a]	-1.41[b]	-1.14[a]	0.0650.003211
12806211	DDR1	discoidin domain receptor tyrosine kinase 1	1.00[a]	1.20[b]	1.04[a]	0.0390.004325
12728696	DDX10	DEAD (Asp-Glu-Ala-Asp) box polypeptide 10	1.00[a]	-1.15[b]	-1.02[a]	0.0210.001767
12861406	DDX23	DEAD (Asp-Glu-Ala-Asp) box polypeptide 23	1.00[a]	-1.03[a]	1.14[b]	0.0340.003180
12793017	DDX24	DEAD (Asp-Glu-Ala-Asp) box polypeptide 24	1.00[a]	1.09[b]	1.14[c]	0.0180.000124

Table 5.5 (continued)

12721139	DEFB122	beta-defensin 122	1.00[a]	1.24[b]	-1.05[a]	0.0400.002239
12730301	DENND5A	DENN	1.00[a]	-1.02[a]	1.10[b]	0.0240.004598
12719581	DHTKD1	dehydrogenase E1 and transketolase domain containing 1	1.00[a]	-1.08[b]	1.01[a]	0.0120.000218
12880985	DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1	1.00[a]	-1.21[b]	1.04[a]	0.0370.002945
12813289	DOC2A	double C2-like domains, alpha	1.00[a]	-1.03[a]	-1.43[b]	0.0680.001517
12706234	DPM2	dolichyl-phosphate mannosyltransferase polypeptide 2, regulator	1.00[a]	1.23[b]	1.21[b]	0.0420.003130
12683999	DSCR3	Down syndrome critical region protein 3	1.00[a]	1.27[b]	1.29[b]	0.0470.002664
12810528	DTNA	dystrobrevin, alpha	1.00[a]	-1.17[b]	-1.11[b]	0.0300.002934
12844671	DUSP23	dual specificity phosphatase 23	1.00[a]	1.12[b]	1.08[b]	0.0220.004302
12765609	EFNB3	ephrin-B3	1.00[a]	1.02[a]	-1.18[b]	0.0220.000204
12753121	EGLN2	egl nine homolog 2 (C. elegans)	1.00[a]	1.08[b]	1.07[b]	0.0150.002486
12761624	EIF4A1	eukaryotic translation initiation factor 4A1	1.00[a]	1.16[b]	1.27[b]	0.0450.004284
12777883	EIF4G3	eukaryotic translation initiation factor 4 gamma, 3	1.00[a]	-1.07[b]	1.03[a]	0.0130.000365
12887939	ELAVL1	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 1 (	1.00[a]	1.03[a]	1.13[b]	0.0180.000510
12910733	ELK1	ELK1, member of ETS oncogene family	1.00[a]	1.15[b]	1.15[b]	0.0240.000991
12740756	ELK4	ELK4, ETS-domain protein (SRF accessory protein 1)	1.00[a]	-1.08[b]	1.09[c]	0.0300.001377

Table 5.5 (continued)

12894451	ENTPD4	ectonucleoside triphosphate diphosphohydrolase 4	1.00[a]	1.20[b]	1.17[b]	0.0270.000244
12783685	EPC2	enhancer of polycomb homolog 2 (Drosophila)	1.00[a]	-1.18[b]	-1.14[b]	0.0200.000161
12776360	EPHA2	EPH receptor A2	1.00[a]	1.20[b]	1.20[b]	0.0420.004164
12850821	EPHB6	EPH receptor B6	1.00[a]	1.30[b]	1.16[c]	0.0410.001008
12796560	ERC2	ELKS	1.00[a]	-1.24[b]	-1.21[b]	0.0420.001783
12789922	ERICH1	glutamate-rich 1	1.00[a]	-1.23[b]	1.07[a]	0.0400.003205
12735710	ERRFI1	ERBB receptor feedback inhibitor 1	1.00[a]	-1.39[b]	-1.17[c]	0.0540.001031
12835715	F3	coagulation factor III (thromboplastin, tissue factor)	1.00[a]	-1.45[b]	-1.03[a]	0.0740.003769
12858708	FAIM2	Fas apoptotic inhibitory molecule 2	1.00[a]	1.15[b]	1.27[c]	0.0370.001604
12905719	FAM127A	family with sequence similarity 127, member A	1.00[a]	1.14[b]	1.17[b]	0.0240.001104
12904144	FAM50A	family with sequence similarity 50, member A	1.00[a]	1.16[b]	1.17[b]	0.0320.002315
12855431	FERD3L	Fer3-like (Drosophila)	1.00[a]	1.18[b]	-1.00[a]	0.0280.003052
12866953	FGFR1OP2	FGFR1 oncogene partner 2	1.00[a]	-1.20[b]	-1.01[a]	0.0300.000464
12771502	FOXK2	forkhead box K2	1.00[a]	1.09[b]	1.12[b]	0.0190.001962
12791616	FURIN	furin (paired basic amino acid cleaving enzyme)	1.00[a]	1.12[b]	1.04[a]	0.0210.001610
12761345	G6PC3	glucose 6 phosphatase, catalytic, 3	1.00[a]	1.13[b]	1.09[b]	0.0170.004554
12767655	GABARAP	GABA(A) receptor-associated protein	1.00[a]	1.08[b]	1.08[b]	0.0170.002289
12893958	GABBR2	gamma-aminobutyric acid (GABA) B receptor, 2	1.00[a]	-1.19[b]	1.04[a]	0.0360.003717

Table 5.5 (continued)

12834169	GAL3ST3	galactose-3-O-sulfotransferase 3	1.00[a]	1.24[b]	1.17[b]	0.0340.003248
12889125	GCNT1	glucosaminyl (N-acetyl) transferase 1, core 2	1.00[a]	-1.38[b]	-1.18[c]	0.0410.000080
12708565	GEN1	Gen homolog 1, endonuclease (Drosophila)	1.00[a]	-1.24[b]	-1.21[b]	0.0460.004141
12885268	GLT25D1	glycosyltransferase 25 domain containing 1	1.00[a]	1.05[a]	1.18[b]	0.0230.000582
12738803	GLTPD1	glycolipid transfer protein domain containing 1	1.00[a]	1.21[b]	1.17[b]	0.0290.000196
12883393	GNB2L1	guanine nucleotide binding protein (G protein), beta po	1.00[a]	1.17[b]	1.05[a]	0.0210.001275
12802708	GNL1	guanine nucleotide binding protein-like 1	1.00[a]	1.17[b]	1.32[c]	0.0470.002236
12803164	GNMT	glycine N-methyltransferase	1.00[a]	1.28[b]	1.22[b]	0.0430.000946
12755952	GOT2	glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate amin	1.00[a]	1.11[b]	1.13[b]	0.0270.003948
12877228	GPX4	glutathione peroxidase 4 (phospholipid hydroperoxidase)	1.00[a]	1.23[b]	1.21[b]	0.0460.003630
12873015	GRID2	glutamate receptor, ionotropic, delta 2	1.00[a]	-1.38[b]	-1.37[b]	0.0470.000396
12724789	GRINA	glutamate receptor, ionotropic, N-methyl D-aspartate-associate	1.00[a]	1.18[b]	1.18[b]	0.0360.002192
12825077	GTF2E2	general transcription factor IIE, polypeptide 2, beta 34kDa	1.00[a]	-1.10[b]	1.02[a]	0.0200.001706
12816165	GTF3C1	general transcription factor IIIC, polypeptide 1, alpha 220kD	1.00[a]	1.16[b]	1.13[b]	0.0250.003275

Table 5.5 (continued)

12703161	GTF3C5	general transcription factor IIIC, polypeptide 5, 63kDa	1.00[a]	1.13[b]	1.16[b]	0.0280.001932
12862551	GXYLT1	glucoside xylosyltransferase 1	1.00[a]	-1.18[b]	-1.06[a]	0.0310.003297
12812382	HBA	hemoglobin, alpha 2	1.00[a]	-1.02[a]	-1.21[b]	0.0280.003829
12780512	HDAC1	histone deacetylase 1	1.00[a]	1.09[b]	1.09[b]	0.0180.001750
12753593	HERPUD1	homocysteine-inducible, endoplasmic reticulum stress-inducib	1.00[a]	1.12[b]	1.19[b]	0.0210.000777
12881065	HIST3H2A	histone cluster 3, H2a	1.00[a]	-1.47[b]	-1.34[b]	0.0520.000539
12714795	HM13	histocompatibility (minor) 13	1.00[a]	1.25[b]	1.19[b]	0.0470.003844
12859274	HMOX1	heme oxygenase (decycling) 1	1.00[a]	1.24[b]	1.21[b]	0.0380.002913
12707032	HNRPLL	heterogeneous nuclear ribonucleoprotein L-like	1.00[a]	-1.12[b]	-1.03[a]	0.0210.002408
12848151	HOOK1	hook homolog 1 (Drosophila)	1.00[a]	-1.22[b]	-1.12[c]	0.0320.000791
12724458	HSF1	heat shock transcription factor 1	1.00[a]	1.16[b]	1.13[b]	0.0290.002434
12900016	HSF2	heat shock transcription factor 2	1.00[a]	-1.29[b]	-1.14[c]	0.0430.001935
12793338	IFI27L2	family with sequence similarity 14, member A	1.00[a]	1.35[b]	1.34[b]	0.0380.000062
12683438	IFNAR1	interferon (alpha, beta and omega) receptor 1	1.00[a]	-1.06[b]	1.06[c]	0.0140.000244
12698826	IL18R1	interleukin 18 receptor 1	1.00[a]	-1.29[b]	-1.17[b]	0.0440.004298
12700517	IL1RL1	interleukin 1 receptor-like 1	1.00[a]	-1.68[b]	-1.11[a]	0.0880.000887
12885382	IL27RA	interleukin 27 receptor, alpha	1.00[a]	1.15[b]	1.20[b]	0.0270.001254
12843689	IL6R	interleukin 6 receptor	1.00[a]	-1.27[b]	-1.10[c]	0.0280.001777
12699444	IMMT	inner membrane protein, mitochondrial	1.00[a]	1.12[b]	1.15[b]	0.0240.001104

Table 5.5 (continued)

12850129	ING3	inhibitor of growth family, member 3	1.00[a]	-1.11[b]	-1.04[a]	0.0210.004107
12757444	IRF3	interferon regulatory factor 3	1.00[a]	1.20[b]	1.19[b]	0.0320.000674
12691723	ISCA2	iron-sulfur cluster assembly 2 homolog (S. cerevisiae)	1.00[a]	-1.16[b]	1.02[a]	0.0330.002982
12768879	ITGA3	integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 recepto	1.00[a]	1.09[a]	1.24[b]	0.0320.002297
12894663	KANK1	KN motif and ankyrin repeat domains 1	1.00[a]	-1.17[b]	-1.05[a]	0.0290.002600
12826028	KCNK1	potassium channel, subfamily K, member 1	1.00[a]	1.33[b]	1.42[b]	0.0730.004316
12890299	KIAA0020	KIAA0020	1.00[a]	1.02[a]	1.20[b]	0.0300.002070
12893285	KIAA1539	btKIAA1539	1.00[a]	1.13[b]	1.13[b]	0.0230.002496
12687612	KLHL33	kelch-like 33 (Drosophila)	1.00[a]	1.17[b]	-1.06[a]	0.0470.003897
12699829	KLRAQ1	KLRAQ motif containing 1	1.00[a]	-1.15[b]	-1.06[c]	0.0250.001191
12840822	KRTCAP2	keratinocyte associated protein 2	1.00[a]	1.25[b]	1.19[b]	0.0420.004089
12872678	LAP3	leucine aminopeptidase 3	1.00[a]	-1.06[a]	1.14[b]	0.0270.000199
12791099	LASS3	LAG1 homolog, ceramide synthase 3	1.00[a]	-1.19[b]	-1.19[b]	0.0240.000248
12817910	LIMK1	LIM domain kinase 1	1.00[a]	1.17[b]	1.11[b]	0.0250.001312
12742621	LIMK2	LIM domain kinase 2	1.00[a]	1.23[b]	1.31[b]	0.0410.002188
12731643	LIN7C	lin-7 homolog C (C. elegans)	1.00[a]	-1.16[b]	-1.05[a]	0.0280.004324
12842993	LMNA	lamin A	1.00[a]	1.15[b]	1.23[b]	0.0410.003505
12733478	LMO2	LIM domain only 2 (rhombotin-like 1)	1.00[a]	1.17[b]	1.00[a]	0.0300.001104
12709937	LNX2	ligand of numb-protein X 2	1.00[a]	1.03[a]	1.18[b]	0.0250.001035

Table 5.5 (continued)

12876135	LOC100124511	hypothetical protein LOC100124511	1.00[a]	-1.15[b]	-1.10[b]	0.0190.001463
12902720	LOC100125231	melanoma antigen family D, 4-like	1.00[a]	1.20[b]	1.17[b]	0.0370.004444
12682890	LOC100139345	similar to mCG67939	1.00[a]	-1.16[b]	-1.02[a]	0.0330.003398
12766595	LOC100139452	similar to Ras-related protein Rab-37	1.00[a]	1.08[a]	-1.16[b]	0.0460.004560
12842284	LOC100139518	hypothetical protein LOC100139518	1.00[a]	1.09[a]	-1.13[b]	0.0390.004535
12692887	LOC100140532	similar to novel ankyrin repeat domain containing prote	1.00[a]	1.03[a]	-1.16[b]	0.0380.004864
12811041	LOC100297063	hypothetical protein LOC100297063	1.00[a]	1.26[b]	1.06[a]	0.0280.000464
12693130	LOC100298870	hypothetical protein LOC100298870	1.00[a]	1.15[a]	-1.40[b]	0.0910.002192
12856434	LOC100300099	hypothetical LOC100300099	1.00[a]	1.05[a]	-1.15[b]	0.0380.004210
12829208	LOC100300295	hypothetical protein LOC100300295	1.00[a]	-1.09[b]	1.07[a]	0.0240.004601
12755419	LOC100300479	similar to zinc finger protein 347	1.00[a]	-1.33[b]	-1.08[a]	0.0400.003708
12902540	LOC100302527	hypothetical protein LOC100302527	1.00[a]	1.22[b]	-1.13[a]	0.0480.001531
12713427	LOC100335935	hypothetical protein LOC100335935	1.00[a]	-1.42[b]	-1.54[b]	0.0930.003451
12774061	LOC100336279	hypothetical protein LOC100336279	1.00[a]	1.08[b]	1.19[c]	0.0240.002733
12906652	LOC100336560	Wiskott-Aldrich syndrome protein-like	1.00[a]	1.12[b]	-1.12[c]	0.0350.002516
12902777	LOC100337433	SRY-box containing gene 3-like	1.00[a]	1.11[b]	-1.04[a]	0.0300.003986

Table 5.5 (continued)

12849524	LOC504276	similar to purine-rich element binding protein B	1.00[a]	-1.15[b]	-1.02[a]	0.0220.002575
12761528	LOC504773	regakine 1	1.00[a]	1.39[b]	1.37[b]	0.0660.003907
12884493	LOC509184	transmembrane emp24 protein transport domain contain	1.00[a]	1.20[b]	1.12[c]	0.0260.000422
12735389	LOC510860	C4b-binding protein alpha-like	1.00[a]	1.32[b]	1.71[c]	0.0810.000751
12705756	LOC514980	similar to Epididymal-specific lipocalin-9 precursor	1.00[a]	1.03[a]	-1.13[b]	0.0230.001007
12735655	LOC515150	similar to C4b-binding protein alpha chain precursor (C4bp) (	1.00[a]	1.12[a]	1.32[b]	0.0530.004827
12822330	LOC518801	similar to CG17687 CG17687-PA	1.00[a]	-1.67[b]	-1.42[b]	0.0830.000545
12892254	LOC521877	ADAM metallopeptidase domain 21	1.00[a]	-1.16[b]	-1.23[b]	0.0330.001347
12879658	LOC522449	hypothetical LOC522449	1.00[a]	-1.20[b]	-1.08[a]	0.0320.003881
12685045	LOC522736	similar to aryl hydrocarbon receptor 2	1.00[a]	1.19[b]	1.02[a]	0.0330.002377
12850554	LOC523019	similar to Sp4 transcription factor	1.00[a]	-1.17[b]	-1.15[b]	0.0190.000531
12689874	LOC523056	similar to BCL2-like 10 (apoptosis facilitator)	1.00[a]	-1.26[b]	-1.30[b]	0.0380.003795
12900759	LOC529052	similar to F55A4.8a	1.00[a]	-1.19[b]	-1.21[b]	0.0370.004408
12710062	LOC532698	hypothetical protein LOC532698	1.00[a]	-1.12[b]	-1.04[a]	0.0250.004882
12686282	LOC539374	similar to Family with sequence similarity 43, membe	1.00[a]	-1.25[b]	-1.24[b]	0.0540.004346

Table 5.5 (continued)

12826933	LOC540046	similar to Protein GCAP14 homolog	1.00[a]	-1.07[b]	-1.04[c]	0.0140.002327
12700376	LOC540073	TER94-like	1.00[a]	-1.14[b]	-1.05[a]	0.0190.002229
12803402	LOC540812	hypothetical LOC540812	1.00[a]	-1.30[b]	-1.15[c]	0.0420.000947
12828800	LOC540918	hypothetical protein LOC540918	1.00[a]	-1.30[b]	-1.16[c]	0.0340.000172
12834845	LOC613830	similar to C11orf61 protein	1.00[a]	-1.11[b]	-1.04[a]	0.0170.001919
12707896	LOC614219	similar to ribosomal protein S23	1.00[a]	-1.01[a]	-1.28[b]	0.0320.002266
12823043	LOC614416	similar to Chondroitin sulfate proteoglycan 5 (neuroglycan	1.00[a]	-1.27[b]	1.02[a]	0.0310.000371
12905745	LOC616201	hypothetical protein	1.00[a]	-1.23[b]	-1.08[a]	0.0290.002123
12737570	LOC617545	similar to hCG19301	1.00[a]	-1.11[b]	-1.17[b]	0.0310.004218
12910436	LOC781486	similar to melanoma antigen family B, 2	1.00[a]	1.05[a]	-1.17[b]	0.0280.000853
12759762	LOC783144	similar to RPL13 protein-like	1.00[a]	1.28[b]	1.09[a]	0.0500.003017
12902133	LOC783232	similar to fertilin alpha	1.00[a]	-1.04[a]	-1.29[b]	0.0410.003357
12862952	LOC783379	KIAA1033-like	1.00[a]	-1.18[b]	-1.13[b]	0.0300.001896
12848053	LOC784007	similar to LOC496253 protein	1.00[a]	-1.22[b]	-1.04[a]	0.0400.003430
12748325	LOC784783	similar to GTP-binding nuclear protein Ran (GTPase Ran) (R	1.00[a]	-1.16[b]	-1.17[b]	0.0300.001113
12784693	LOC786771	similar to 40S ribosomal protein SA (p40) (34	1.00[a]	1.21[b]	-1.04[a]	0.0480.004530
12882396	LOC787836	similar to EGF-like module-containing mucin-like hormone r	1.00[a]	1.52[b]	1.14[a]	0.0700.001167
12882506	LOC789554	similar to olfactory receptor, family 7, subfamily A, memb	1.00[a]	1.12[b]	-1.07[a]	0.0260.003809

Table 5.5 (continued)

12745320	LRAT	lecithin retinol acyltransferase (phosphatidylcholine--retinol O-a	1.00[a]	-1.13[b]	1.18[c]	0.0340.000322
12831438	LRP5	low density lipoprotein receptor-related protein 5	1.00[a]	1.20[b]	1.11[c]	0.0290.003349
12861337	LRP6	low density lipoprotein receptor-related protein 6	1.00[a]	-1.11[b]	1.01[a]	0.0260.003775
12877145	LRRC25	leucine rich repeat containing 25	1.00[a]	1.26[b]	1.10[a]	0.0390.002285
12846866	LRRC8D	leucine rich repeat containing 8 family, member D	1.00[a]	-1.08[b]	1.08[c]	0.0220.002475
12867385	LRRK2	leucine-rich repeat kinase 2	1.00[a]	-1.27[b]	-1.14[c]	0.0380.001168
12804382	LTA	lymphotoxin alpha (TNF superfamily, member 1)	1.00[a]	1.14[b]	-1.07[a]	0.0390.003022
12784665	LYPLA2	lysophospholipase II	1.00[a]	1.24[b]	1.15[c]	0.0260.000027
12688919	LYSMD2	LysM, putative peptidoglycan-binding, domain containing 2	1.00[a]	1.27[b]	1.19[b]	0.0430.001660
12846097	MAGI3	membrane associated guanylate kinase, WW and PDZ domain contai	1.00[a]	1.06[a]	1.19[b]	0.0310.004709
12879542	MAN2A1	mannosidase, alpha, class 2A, member 1	1.00[a]	-1.06[a]	1.07[b]	0.0300.004885
12788277	MARVELD2	MARVEL domain containing 2	1.00[a]	-1.07[a]	1.09[b]	0.0270.002638
12886733	MAST3	microtubule associated serine	1.00[a]	1.20[b]	1.09[c]	0.0270.001472
12721094	MAVS	mitochondrial antiviral signaling protein	1.00[a]	1.07[b]	1.13[c]	0.0180.002325
12877798	MCOLN1	mucolipin 1	1.00[a]	1.27[b]	1.10[c]	0.0290.000309

Table 5.5 (continued)

12705668	MFSD9	major facilitator superfamily domain containing 9	1.00[a]	-1.33[b]	-1.07[a]	0.0410.002199
12899003	MGC137021	hypothetical protein MGC137021	1.00[a]	-1.41[b]	-1.07[a]	0.0470.000875
12714218	MGC138976	hypothetical LOC506205	1.00[a]	-1.24[b]	-1.04[a]	0.0350.002616
12891753	MGC140340	RMI1, RecQ mediated genome instability 1, homolog (S. cere	1.00[a]	-1.21[b]	1.11[a]	0.0500.004331
12707543	MGC142792	similar to Lims E protein	1.00[a]	-1.10[b]	1.02[a]	0.0250.003458
12694035	MGC152585	hypothetical LOC507035	1.00[a]	-1.09[b]	-1.02[a]	0.0170.004624
12696838	MGC159550	hypothetical LOC540184	1.00[a]	-1.32[b]	-1.13[c]	0.0360.000227
12747213	MGC165715	hypothetical LOC530484	1.00[a]	-1.24[b]	-1.25[b]	0.0390.001040
12711935	MGC165939	Uncharacterized protein C13orf18 homolog	1.00[a]	-1.52[b]	-1.15[a]	0.0420.000201
12688769	MGC166084	hypothetical LOC509393	1.00[a]	-1.13[b]	-1.00[a]	0.0160.000579
12879792	MIDN	midnolin	1.00[a]	1.26[b]	1.32[b]	0.0490.001746
12698235	MIR1301	microRNA mir-1301	1.00[a]	-1.29[b]	-1.04[a]	0.0320.000028
12683006	MIR155	microRNA mir-155	1.00[a]	-1.18[b]	-1.13[b]	0.0220.001071
12791562	MIR211	microRNA mir-211	1.00[a]	1.03[a]	-1.14[b]	0.0330.002774
12708822	MIR2303	microRNA mir-2303	1.00[a]	-1.14[b]	-1.10[b]	0.0230.001388
12794243	MIR2372	microRNA mir-2372	1.00[a]	-1.09[a]	1.21[b]	0.0470.001670
12842451	MIR2416	microRNA mir-2416	1.00[a]	1.06[a]	1.29[b]	0.0280.000081
12892478	MIR2473	microRNA mir-2473	1.00[a]	-1.32[b]	-1.02[a]	0.0590.002304
12679724	MIS18A	protein Mis18-alpha	1.00[a]	1.16[b]	-1.04[a]	0.0170.000292
12859364	MLF2	myeloid leukemia factor 2	1.00[a]	1.16[b]	1.17[b]	0.0340.003484
12846912	MLLT11	myeloid	1.00[a]	1.23[b]	1.36[b]	0.0550.004406
12721755	MMP16	matrix metallopeptidase 16 (membrane-inserted)	1.00[a]	-1.25[b]	-1.05[a]	0.0250.000112

Table 5.5 (continued)

12732358	MPZL3	myelin protein zero-like 3	1.00[a]	-1.12[b]	-1.02[a]	0.0220.004128
12818395	MRPS34	mitochondrial ribosomal protein S34	1.00[a]	1.22[b]	1.12[c]	0.0340.001237
12896461	MTHFD1L	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-	1.00[a]	1.19[b]	1.33[b]	0.0420.002110
12710450	MTMR6	myotubularin related protein 6	1.00[a]	-1.09[b]	-1.09[b]	0.0160.003962
12697268	MTX3	metaxin 3	1.00[a]	-1.25[b]	-1.19[b]	0.0370.000538
12745765	MVK	mevalonate kinase	1.00[a]	1.04[b]	-1.04[c]	0.0150.001540
12896715	MYB	v-myb myeloblastosis viral oncogene homolog (avian)	1.00[a]	-1.29[b]	-1.01[a]	0.0470.001758
12686285	MYNN	myoneurin	1.00[a]	-1.12[b]	-1.01[a]	0.0270.003876
12710371	NAA16	N(alpha)-acetyltransferase 16, NatA auxiliary subunit	1.00[a]	-1.14[b]	-1.08[b]	0.0260.002728
12830931	NAA40	N(alpha)-acetyltransferase 40, NatD catalytic subunit, homolog	1.00[a]	1.19[b]	1.22[b]	0.0390.002047
12781710	NAB1	NGFI-A binding protein 1 (EGR1 binding protein 1)	1.00[a]	-1.11[b]	1.06[a]	0.0290.001415
12864496	NAB2	NGFI-A binding protein 2 (EGR1 binding protein 2)	1.00[a]	1.25[b]	1.42[b]	0.0730.004579
12854527	NAMPT	nicotinamide phosphoribosyltransferase	1.00[a]	-1.19[b]	-1.06[a]	0.0260.000542
12891577	NANS	N-acetylneuraminic acid synthase	1.00[a]	1.14[b]	1.19[b]	0.0340.004953
12883428	NDUFA2	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2, 8kDa	1.00[a]	1.16[b]	1.18[b]	0.0310.003240
12815307	NDUFAB1	NADH dehydrogenase (ubiquinone) 1, alpha	1.00[a]	1.21[b]	1.12[c]	0.0270.001157

Table 5.5 (continued)

12774890	NEB	nebulin	1.00[a]	-1.17[b]	-1.17[b]	0.0330.003038
12887575	NFIC	nuclear factor I	1.00[a]	1.11[b]	1.12[b]	0.0200.003140
12791746	NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-	1.00[a]	-1.44[b]	-1.24[b]	0.0650.004582
12803223	NFYA	nuclear transcription factor Y, alpha	1.00[a]	1.03[a]	1.10[b]	0.0150.001019
12775945	NINJ1	ninjurin 1	1.00[a]	1.21[b]	1.14[b]	0.0320.003704
12815983	NLRC3	NLR family, CARD domain containing 3	1.00[a]	1.04[a]	-1.17[b]	0.0340.000468
12851927	NME1-NME2	NME1-NME2 readthrough	1.00[a]	1.36[b]	1.26[b]	0.0500.000390
12910154	NONO	non-POU domain containing, octamer-binding	1.00[a]	1.08[b]	1.03[a]	0.0110.003000
12718233	NOP56	NOP56 ribonucleoprotein homolog (yeast)	1.00[a]	1.09[b]	1.12[b]	0.0220.004132
12752495	NPAS1	neuronal PAS domain protein 1	1.00[a]	1.14[b]	-1.12[c]	0.0320.001194
12713592	NPBWR2	neuropeptides B	1.00[a]	1.01[a]	-1.17[b]	0.0340.004519
12679345	NPHP3	nephronophthisis 3 (adolescent)	1.00[a]	-1.15[b]	-1.08[c]	0.0250.003137
12821454	NT5C2	5'-nucleotidase, cytosolic II	1.00[a]	-1.19[b]	-1.16[b]	0.0340.004432
12791125	NUBPL	nucleotide binding protein-like	1.00[a]	-1.21[b]	-1.14[b]	0.0400.002994
12794079	NUP210	nucleoporin 210	1.00[a]	1.12[b]	1.01[a]	0.0140.001641
12858331	NUP50	nucleoporin 50kDa	1.00[a]	-1.02[a]	1.16[b]	0.0210.000159
12753711	NUTF2	nuclear transport factor 2	1.00[a]	1.07[b]	1.10[b]	0.0200.003096
12836937	OMA1	OMA1 homolog, zinc metallopeptidase (S. cerevisiae)	1.00[a]	-1.37[b]	-1.18[c]	0.0580.002711

Table 5.5 (continued)

12686108	OSBPL11	oxysterol binding protein-like 11	1.00[a]	-1.26[b]	-1.10[c]	0.0200.000218
12851380	OSBPL3	oxysterol binding protein-like 3	1.00[a]	1.09[a]	1.38[b]	0.0550.004536
12776601	OSGEPL1	O-sialoglycoprotein endopeptidase-like 1	1.00[a]	-1.31[b]	-1.01[a]	0.0320.000349
12731077	P4HA3	prolyl 4-hydroxylase, alpha polypeptide III	1.00[a]	-1.36[b]	-1.08[a]	0.0570.003208
12832821	PAG11	pregnancy-associated glycoprotein 11	1.00[a]	-1.34[b]	-1.32[b]	0.0590.003888
12882286	PALM	paralemmin	1.00[a]	1.15[b]	-1.02[a]	0.0350.004348
12787282	PANK3	pantothenate kinase 3	1.00[a]	-1.17[b]	-1.00[a]	0.0340.001657
12884529	PCDH1	protocadherin 1	1.00[a]	1.12[b]	1.08[b]	0.0160.000485
12876146	PCDHGC3	protocadherin gamma subfamily C, 3	1.00[a]	1.27[b]	1.21[b]	0.0410.000861
12693599	PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	1.00[a]	1.19[b]	1.12[b]	0.0270.003696
12825212	PCM1	pericentriolar material 1	1.00[a]	-1.12[b]	-1.07[b]	0.0220.003172
12853639	PDK4	pyruvate dehydrogenase kinase, isozyme 4	1.00[a]	-1.76[b]	-1.47[b]	0.1190.003779
12761334	PEMT	phosphatidylethanolamine N-methyltransferase	1.00[a]	1.16[b]	1.10[b]	0.0280.004954
12682676	PFN1	profilin 1	1.00[a]	1.23[b]	1.24[b]	0.0460.002123
12732120	PGM2L1	phosphoglucomutase 2-like 1	1.00[a]	-1.40[b]	-1.41[b]	0.0670.002707
12812393	PHKG2	phosphorylase kinase, gamma 2 (testis)	1.00[a]	1.15[b]	1.09[b]	0.0240.002756
12860985	PICK1	protein interacting with PRKCA 1	1.00[a]	1.19[b]	1.24[b]	0.0330.000841
12772901	PITPNA	phosphatidylinositol transfer protein, alpha	1.00[a]	1.13[b]	1.20[b]	0.0350.003281

Table 5.5 (continued)

12771785	PMP22	peripheral myelin protein 22	1.00[a]	-1.16[b]	-1.31[c]	0.0470.001497
12819454	PNLIPRP2	pancreatic lipase-related protein 2	1.00[a]	1.13[b]	-1.02[a]	0.0160.002468
12779481	POLR2D	polymerase (RNA) II (DNA directed) polypeptide D	1.00[a]	1.05[a]	1.24[b]	0.0280.000124
12702934	POMT1	protein-O-mannosyltransferase 1	1.00[a]	1.18[b]	1.11[b]	0.0310.002608
12724871	POP1	processing of precursor 1, ribonuclease P	1.00[a]	1.10[b]	1.24[c]	0.0340.002457
12813437	POR	P450 (cytochrome) oxidoreductase	1.00[a]	1.14[b]	1.18[b]	0.0340.003956
12866906	PPHLN1	periphilin 1	1.00[a]	-1.11[b]	1.01[a]	0.0280.004763
12844589	PPIH	peptidylprolyl isomerase H (cyclophilin H)	1.00[a]	1.11[b]	1.20[b]	0.0340.003938
12833867	PPP1CA	protein phosphatase 1, catalytic subunit, alpha isozyme	1.00[a]	1.16[b]	1.15[b]	0.0290.002950
12697381	PPP1R14D	protein phosphatase 1, regulatory (inhibitor) subunit 14D	1.00[a]	1.07[a]	1.22[b]	0.0270.000926
12687438	PPP2R3A	protein phosphatase 2 (formerly 2A), regulatory subunit B",	1.00[a]	-1.13[b]	-1.01[a]	0.0250.002523
12809449	PPP4R1	protein phosphatase 4, regulatory subunit 1	1.00[a]	1.04[a]	1.13[b]	0.0140.002877
12798367	PPP4R2	protein phosphatase 4, regulatory subunit 2	1.00[a]	-1.19[b]	-1.01[a]	0.0300.000689
12896699	PREP	prolyl endopeptidase	1.00[a]	-1.16[b]	-1.00[a]	0.0310.003455
12798533	PRKCD	protein kinase C, delta	1.00[a]	1.25[b]	1.25[b]	0.0430.001261
12757145	PRPF31	PRP31 pre-mRNA processing factor 31 homolog (S. cerevis	1.00[a]	1.22[b]	1.10[c]	0.0300.000416

Table 5.5 (continued)

12843627	PRPF38A	PRP38 pre-mRNA processing factor 38 (yeast) domain containin	1.00[a]	-1.14[b]	1.02[a]	0.0330.004499
12805379	PRPF4B	PRP4 pre-mRNA processing factor 4 homolog B (yeast)	1.00[a]	-1.06[b]	1.03[a]	0.0130.004034
12813522	PRR14	proline rich 14	1.00[a]	1.19[b]	1.18[b]	0.0280.003635
12817104	PRSS27	protease, serine 27	1.00[a]	-1.00[a]	-1.22[b]	0.0360.000775
12691536	PRTG	protogenin	1.00[a]	-1.38[b]	-1.00[a]	0.0560.000335
12749769	PSENEN	presenilin enhancer 2 homolog (C. elegans)	1.00[a]	1.24[b]	1.13[c]	0.0260.000202
12751335	PSMD8	proteasome (prosome, macropain) 26S subunit, non-ATPase, 8	1.00[a]	1.23[b]	1.14[b]	0.0360.001973
12808197	PSMG2	proteasome (prosome, macropain) assembly chaperone 2	1.00[a]	1.12[b]	1.23[c]	0.0390.002869
12819493	PSTK	phosphoseryl-tRNA kinase	1.00[a]	-1.45[b]	-1.18[c]	0.0510.000141
12809935	PSTPIP2	proline-serine-threonine phosphatase interacting protein 2	1.00[a]	-1.18[b]	1.08[a]	0.0480.004152
12698053	PUM2	pumilio homolog 2 (Drosophila)	1.00[a]	-1.15[b]	-1.05[a]	0.0210.001237
12707977	PUS10	pseudouridylate synthase 10	1.00[a]	-1.21[b]	-1.11[c]	0.0300.000513
12818183	QPRT	quinolinate phosphoribosyltransferase	1.00[a]	1.16[b]	1.17[b]	0.0240.001686
12843452	RAB25	RAB25, member RAS oncogene family	1.00[a]	1.28[b]	1.29[b]	0.0510.002290
12748015	RAB35	RAB35, member RAS oncogene family	1.00[a]	1.15[b]	1.17[b]	0.0310.002854
12882589	RAB8A	RAB8A, member RAS oncogene family	1.00[a]	1.11[b]	1.17[b]	0.0300.002103

Table 5.5 (continued)

12705580	RALGDS	ral guanine nucleotide dissociation stimulator	1.00[a]	1.18[b]	1.22[b]	0.0390.001348
12842709	RAP1A	RAP1A, member of RAS oncogene family	1.00[a]	-1.17[b]	-1.02[a]	0.0290.002002
12762096	RASD1	RAS, dexamethasone-induced 1	1.00[a]	-1.68[b]	-1.34[c]	0.0840.001633
12893594	RASEF	RAS and EF-hand domain containing	1.00[a]	-1.46[b]	-1.13[a]	0.0430.002794
12873681	RASGEF1B	RasGEF domain family, member 1B	1.00[a]	-1.37[b]	-1.18[c]	0.0400.000074
12867519	RASSF3	Ras association (RalGDS	1.00[a]	-1.30[b]	-1.14[c]	0.0380.000443
12722010	RBM12B	RNA binding motif protein 12B	1.00[a]	-1.14[b]	-1.07[c]	0.0250.004241
12849958	RBM33	RNA binding motif protein 33	1.00[a]	1.05[a]	-1.07[b]	0.0230.004934
12907794	RBMX	RNA binding motif protein, X-linked	1.00[a]	-1.11[b]	-1.05[c]	0.0210.002635
12875984	RELL1	RELT-like 1	1.00[a]	-1.01[a]	1.18[b]	0.0330.001447
12901006	REPS1	RALBP1 associated Eps domain containing 1	1.00[a]	-1.19[b]	-1.08[c]	0.0230.001435
12693205	RHEB	Ras homolog enriched in brain	1.00[a]	1.09[b]	1.09[b]	0.0190.003562
12839908	RIT1	Ras-like without CAAX 1	1.00[a]	-1.24[b]	-1.05[a]	0.0220.000106
12879151	RMND5B	required for meiotic nuclear division 5 homolog B (S. cerevis	1.00[a]	1.19[b]	1.10[c]	0.0240.000397
12841126	RNF115	ring finger protein 115	1.00[a]	-1.07[b]	1.04[a]	0.0130.002442
12705891	RNF181	ring finger protein 181	1.00[a]	1.13[b]	1.15[b]	0.0200.001115
12698562	ROCK2	Rho-associated, coiled-coil containing protein kinase 2	1.00[a]	-1.12[b]	-1.03[a]	0.0260.004939
12757517	RPGRIP1L	RPGRIP1-like	1.00[a]	-1.22[b]	-1.11[c]	0.0320.002587

Table 5.5 (continued)

12697019	RPL36AL	ribosomal protein L36a-like	1.00[a]	1.13[b]	1.02[a]	0.0170.003333
12802388	RPL7L1	ribosomal protein L7-like 1	1.00[a]	1.13[b]	1.12[b]	0.0230.001063
12743489	RSRC2	arginine	1.00[a]	-1.15[b]	-1.08[c]	0.0210.000678
12689648	RTF1	Rtf1, Paf1	1.00[a]	-1.06[b]	1.01[a]	0.0140.003807
12765283	RUNDC3A	RUN domain containing 3A	1.00[a]	1.19[b]	1.23[b]	0.0410.001828
12825048	RWDD4	RWD domain containing 4	1.00[a]	-1.14[b]	1.02[a]	0.0240.002495
12695025	SAV1	salvador homolog 1 (Drosophila)	1.00[a]	-1.13[b]	1.04[a]	0.0380.004911
12843873	SCP2	sterol carrier protein 2	1.00[a]	-1.21[b]	-1.25[b]	0.0460.004580
12726044	SDC2	syndecan 2	1.00[a]	1.02[a]	1.21[b]	0.0390.003408
12774661	SDHB	succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	1.00[a]	1.12[b]	1.03[a]	0.0250.003311
12782736	SDPR	serum deprivation response	1.00[a]	-1.17[b]	-1.18[b]	0.0340.003571
12798942	SEC13	SEC13 homolog (S. cerevisiae)	1.00[a]	1.24[b]	1.19[b]	0.0410.001450
12770064	SEC14L1	SEC14-like 1 (S. cerevisiae)	1.00[a]	1.09[a]	1.26[b]	0.0390.002855
12742060	SEC14L2	SEC14-like 2 (S. cerevisiae)	1.00[a]	1.26[b]	1.32[b]	0.0410.000822
12826094	SEC24C	SEC24 family, member C (S. cerevisiae)	1.00[a]	1.08[b]	1.12[b]	0.0160.002926
12870423	SEC24D	SEC24 family, member D (S. cerevisiae)	1.00[a]	1.21[b]	1.18[b]	0.0340.001266
12798378	SEC61A1	Sec61 alpha 1 subunit (S. cerevisiae)	1.00[a]	1.24[b]	1.22[b]	0.0410.002461
12761154	SERPINF2	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigm	1.00[a]	-1.02[a]	-1.21[b]	0.0220.000315

Table 5.5 (continued)

12727477	SERPINH1	serpin peptidase inhibitor, clade H (heat shock protein 47)	1.00[a]	1.22[b]	1.33[b]	0.0400.000301
12779743	SESTD1	SEC14 and spectrin domains 1	1.00[a]	-1.18[b]	-1.08[c]	0.0280.003866
12811815	SEZ6L2	seizure related 6 homolog (mouse)-like 2	1.00[a]	1.17[b]	1.08[c]	0.0320.004676
12795342	SFMBT1	Scm-like with four mbt domains 1	1.00[a]	-1.07[b]	1.09[c]	0.0280.002702
12899008	SFRS18	splicing factor, arginine	1.00[a]	-1.14[b]	-1.08[c]	0.0260.003542
12819502	SFXN3	sideroflexin 3	1.00[a]	1.25[b]	1.29[b]	0.0420.000607
12761383	SHBG	sex hormone-binding globulin	1.00[a]	1.16[b]	1.11[b]	0.0170.002561
12864912	SHMT2	serine hydroxymethyltransferase 2 (mitochondrial)	1.00[a]	1.29[b]	1.22[b]	0.0410.000848
12790177	SIN3A	SIN3 homolog A, transcription regulator (yeast)	1.00[a]	1.11[b]	1.11[b]	0.0160.000442
12710363	SLAIN1	SLAIN motif family, member 1	1.00[a]	-1.26[b]	-1.07[a]	0.0360.000341
12769217	SLC25A39	solute carrier family 25, member 39	1.00[a]	1.17[b]	1.20[b]	0.0370.001891
12696210	SLC27A2	solute carrier family 27 (fatty acid transporter), member 2	1.00[a]	1.08[a]	-1.15[b]	0.0390.002407
12758460	SLC27A5	solute carrier family 27 (fatty acid transporter), member 5	1.00[a]	1.15[b]	1.00[a]	0.0270.004638
12729666	SLC35C1	solute carrier family 35, member C1	1.00[a]	1.26[b]	1.42[b]	0.0500.000826
12900723	SLC35F1	solute carrier family 35, member F1	1.00[a]	-1.52[b]	-1.26[c]	0.0540.003264
12691151	SLC39A9	solute carrier family 39 (zinc transporter), member 9	1.00[a]	1.03[a]	1.17[b]	0.0270.004127

Table 5.5 (continued)

12739370	SLC45A1	solute carrier family 45, member 1	1.00[a]	1.15[b]	1.21[b]	0.0260.004130
12722615	SLC45A4	solute carrier family 45, member 4	1.00[a]	1.14[b]	-1.01[a]	0.0170.000066
12777931	SLC4A3	solute carrier family 4, anion exchanger, member 3	1.00[a]	1.21[b]	1.16[b]	0.0360.002173
12798802	SLC4A7	solute carrier family 4, sodium bicarbonate cotransporter, me	1.00[a]	-1.23[b]	-1.09[c]	0.0310.000333
12867619	SLC4A8	solute carrier family 4, sodium bicarbonate cotransport	1.00[a]	-1.16[b]	-1.02[a]	0.0300.001077
12683494	SLC5A3	solute carrier family 5 (inositol transporters), member 3	1.00[a]	-1.13[b]	1.00[a]	0.0240.004110
12883870	SLC5A5	solute carrier family 5 (sodium iodide symporter), memb	1.00[a]	1.04[a]	-1.18[b]	0.0370.002054
12864073	SLC6A15	solute carrier family 6 (neutral amino acid transporter), membe	1.00[a]	1.21[b]	-1.16[a]	0.0520.003426
12843025	SLC6A17	solute carrier family 6, member 17	1.00[a]	1.32[b]	1.11[c]	0.0370.001031
12700968	SLC9A2	solute carrier family 9 (sodium	1.00[a]	-1.30[b]	1.05[a]	0.0390.000107
12820519	SLK	STE20-like kinase	1.00[a]	-1.15[b]	-1.06[c]	0.0220.000793
12822402	SMNDC1	survival motor neuron domain containing 1	1.00[a]	-1.16[b]	-1.04[a]	0.0310.003826
12895130	SMU1	smu-1 suppressor of mec-8 and unc-52 homolog (C. elegans)	1.00[a]	-1.13[b]	-1.05[c]	0.0160.002719

Table 5.5 (continued)

12761769	SNF8	SNF8, ESCRT-II complex subunit, homolog (S. cerevisiae)	1.00[a]	1.12[b]	1.12[b]	0.0250.001929
12705102	SNRNP200	small nuclear ribonucleoprotein 200kDa (U5)	1.00[a]	1.13[b]	1.16[b]	0.0240.001534
12681751	SPATA16	spermatogenesis associated 16	1.00[a]	-1.51[b]	-1.59[b]	0.1040.003659
12880047	SPINK5	serine peptidase inhibitor, Kazal type 5	1.00[a]	-1.07[a]	-1.20[b]	0.0300.001911
12690591	SPINT1	serine peptidase inhibitor, Kunitz type 1	1.00[a]	1.13[b]	1.14[b]	0.0300.004018
12809817	SPIRE1	spire homolog 1 (Drosophila)	1.00[a]	-1.09[b]	1.06[c]	0.0160.000004
12781179	SPOPL	speckle-type POZ protein-like	1.00[a]	-1.19[b]	-1.09[c]	0.0270.002507
12738745	SPSB1	splA	1.00[a]	1.08[a]	1.25[b]	0.0440.004244
12736354	SRP9	signal recognition particle 9kDa	1.00[a]	-1.21[b]	-1.06[a]	0.0370.002371
12861559	SRSF2IP	serine	1.00[a]	-1.14[b]	-1.04[a]	0.0170.000956
12716125	SRXN1	sulfiredoxin 1	1.00[a]	1.20[b]	1.51[c]	0.0720.003800
12837024	SSR2	signal sequence receptor, beta (translocon-associated protein b	1.00[a]	1.18[b]	1.14[b]	0.0260.001949
12903267	SSR4	signal sequence receptor, delta (translocon-associated protein	1.00[a]	1.20[b]	1.10[c]	0.0280.002520
12704183	ST6GALNAC2	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-ac	1.00[a]	1.18[b]	1.19[b]	0.0340.002065
12852887	ST7	suppression of tumorigenicity 7	1.00[a]	1.14[b]	1.13[b]	0.0290.004896

Table 5.5 (continued)

12809232	ST8SIA5	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 5	1.00[a]	1.22[b]	1.22[b]	0.0420.003404
12718263	ST8SIA6	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 6	1.00[a]	-1.39[b]	-1.25[b]	0.0670.004024
12768925	STAT3	signal transducer and activator of transcription 3 (acute-phas	1.00[a]	1.05[a]	1.19[b]	0.0320.003168
12719219	STAU1	staufen, RNA binding protein, homolog 1 (Drosophila)	1.00[a]	-1.03[a]	1.08[b]	0.0150.001211
12831915	STIP1	stress-induced-phosphoprotein 1 (Hsp70	1.00[a]	1.10[b]	1.18[c]	0.0350.003472
12776910	STK16	serine	1.00[a]	1.19[b]	1.15[b]	0.0320.002165
12838532	STK40	serine	1.00[a]	1.11[b]	1.21[c]	0.0280.002233
12719749	STX16	syntaxin 16	1.00[a]	-1.24[b]	-1.11[c]	0.0270.001370
12891106	STX17	syntaxin 17	1.00[a]	-1.16[b]	-1.08[c]	0.0230.003178
12898392	STXBP5	syntaxin binding protein 5 (tomosyn)	1.00[a]	-1.15[b]	-1.05[a]	0.0180.003925
12746819	SUHW2	suppressor of hairy wing homolog 2	1.00[a]	1.10[a]	1.87[b]	0.1020.002525
12678716	SYNJ1	synaptojanin 1	1.00[a]	-1.13[b]	-1.07[c]	0.0120.000062
12695481	SYNJ2BP	synaptojanin 2 binding protein	1.00[a]	-1.07[b]	1.02[a]	0.0110.003501
12875470	SYNPO2	synaptopodin 2	1.00[a]	-1.46[b]	-1.38[b]	0.0680.003691
12831287	TAF6L	TAF6-like RNA polymerase II, p300	1.00[a]	1.17[b]	1.17[b]	0.0220.000169
12764103	TANC2	tetratricopeptide repeat, ankyrin repeat and coiled-coil conta	1.00[a]	1.17[b]	1.11[b]	0.0250.001075

Table 5.5 (continued)

12806911	TAP2	transporter 2, ATP-binding cassette, sub-family B (MDR	1.00[a]	-1.35[b]	-1.38[b]	0.0560.003005
12845308	TCTEX1D1	Tctex1 domain containing 1	1.00[a]	-1.40[b]	-1.11[a]	0.0640.004251
12827943	TFAM	transcription factor A, mitochondrial	1.00[a]	-1.20[b]	-1.09[c]	0.0300.002470
12755803	TGFB1	transforming growth factor, beta 1	1.00[a]	1.30[b]	1.46[b]	0.0480.000291
12825460	THAP1	THAP domain containing, apoptosis associated protein 1	1.00[a]	-1.26[b]	-1.10[c]	0.0310.000187
12780012	THSD7B	thrombospondin, type I, domain containing 7B-like	1.00[a]	-1.52[b]	-1.26[c]	0.0510.000539
12686567	TIPARP	TCDD-inducible poly(ADP-ribose) polymerase	1.00[a]	-1.63[b]	-1.24[c]	0.0780.000537
12709691	TM9SF2	transmembrane 9 superfamily member 2	1.00[a]	1.11[b]	1.07[b]	0.0170.002635
12818583	TMC7	transmembrane channel-like 7	1.00[a]	-1.48[b]	-1.10[a]	0.0610.000428
12770490	TMEM101	transmembrane protein 101	1.00[a]	1.13[b]	1.09[b]	0.0260.004874
12830423	TMEM179B	transmembrane protein 179B	1.00[a]	1.14[b]	1.03[a]	0.0210.000600
12737891	TMEM9	transmembrane protein 9	1.00[a]	1.23[b]	1.15[b]	0.0300.001120
12690496	TMOD3	tropomodulin 3 (ubiquitous)	1.00[a]	-1.17[b]	-1.02[a]	0.0210.000091
12885622	TMPRSS9	transmembrane protease, serine 9	1.00[a]	1.02[a]	-1.20[b]	0.0430.002912
12833169	TNNI2	troponin I type 2 (skeletal, fast)	1.00[a]	1.11[b]	-1.13[c]	0.0360.002979
12856205	TPK1	thiamin pyrophosphokinase 1	1.00[a]	-1.15[b]	-1.13[b]	0.0270.001791
12682407	TRA2B	transformer 2 beta homolog (Drosophila)	1.00[a]	-1.09[b]	1.00[a]	0.0140.000534
12800251	TRH	thyrotropin-releasing hormone	1.00[a]	-1.19[b]	-1.07[a]	0.0310.001559

Table 5.5 (continued)

12696584	TRIM36	tripartite motif-containing 36	1.00[a]	-1.22[b]	-1.00[a]	0.0280.000671
12730210	TRIM44	tripartite motif-containing 44	1.00[a]	-1.13[b]	1.01[a]	0.0190.001088
12706838	TSC1	tuberous sclerosis 1	1.00[a]	-1.15[b]	-1.10[b]	0.0170.000680
12786004	TTC33	tetratricopeptide repeat domain 33	1.00[a]	-1.24[b]	-1.29[b]	0.0510.001320
12707414	TUBB2C	tubulin, beta 2C	1.00[a]	1.18[b]	1.27[b]	0.0470.003575
12679811	TXLNA	taxilin alpha	1.00[a]	1.03[a]	1.16[b]	0.0350.003683
12837120	TXNDC12	thioredoxin domain containing 12 (endoplasmic reticulum)	1.00[a]	1.09[b]	1.10[b]	0.0200.002815
12801023	TXNDC5	thioredoxin domain containing 5 (endoplasmic reticulum)	1.00[a]	1.26[b]	1.34[b]	0.0500.001771
12837074	TXNIP	thioredoxin interacting protein	1.00[a]	-1.48[b]	-1.35[b]	0.0560.000389
12857179	TXNRD1	thioredoxin reductase 1	1.00[a]	-1.04[a]	1.12[b]	0.0240.000666
12748228	UBE2L3	ubiquitin-conjugating enzyme E2L 3	1.00[a]	1.07[b]	1.12[b]	0.0160.002914
12759033	UBE2M	ubiquitin-conjugating enzyme E2M (UBC12 homolog, yeast)	1.00[a]	1.13[b]	1.21[b]	0.0320.001375
12849657	UBE3C	ubiquitin protein ligase E3C	1.00[a]	-1.18[b]	1.01[a]	0.0250.000117
12767886	UBTF	upstream binding transcription factor, RNA polymerase I	1.00[a]	1.02[a]	1.10[b]	0.0140.003852
12846641	UFC1	ubiquitin-fold modifier conjugating enzyme 1	1.00[a]	1.13[b]	1.08[b]	0.0230.003688
12884383	UIMC1	ubiquitin interaction motif containing 1	1.00[a]	-1.11[b]	1.03[a]	0.0220.002515
12732418	USP2	ubiquitin specific peptidase 2	1.00[a]	-1.43[b]	-1.11[a]	0.0520.000622

Table 5.5 (continued)

12764643	USP32	ubiquitin specific peptidase 32	1.00[a]	-1.09[b]	1.04[a]	0.0210.000580
12874296	USP46	ubiquitin specific peptidase 46	1.00[a]	-1.17[b]	1.00[a]	0.0290.001474
12789378	UTP14A	UTP14, U3 small nucleolar ribonucleoprotein, homolog A (yeast	1.00[a]	-1.25[b]	-1.16[b]	0.0450.003745
12876570	VAV1	vav 1 guanine nucleotide exchange factor	1.00[a]	1.15[b]	-1.01[a]	0.0350.003709
12861191	VEZT	vezatin, adherens junctions transmembrane protein	1.00[a]	-1.16[b]	-1.05[a]	0.0210.000873
12818366	VGF	VGF nerve growth factor inducible	1.00[a]	1.48[b]	1.17[a]	0.0680.001709
12800230	VHL	von Hippel-Lindau tumor suppressor	1.00[a]	-1.12[b]	-1.00[a]	0.0210.001920
12797505	VPRBP	Vpr (HIV-1) binding protein	1.00[a]	1.00[a]	1.14[b]	0.0190.000268
12759936	VSIG10L	V-set and immunoglobulin domain containing 10 like	1.00[a]	1.00[a]	-1.20[b]	0.0320.002217
12828515	WAPAL	wings apart-like homolog (Drosophila)	1.00[a]	-1.05[b]	1.07[c]	0.0150.000234
12797242	WDR48	WD repeat domain 48	1.00[a]	-1.11[b]	-1.12[b]	0.0230.002130
12845185	WDR63	WD repeat domain 63	1.00[a]	-1.32[b]	-1.22[c]	0.0260.000016
12788246	WDR70	WD repeat domain 70	1.00[a]	-1.08[b]	1.06[c]	0.0200.000388
12699220	WDR85	WD repeat domain 85	1.00[a]	1.19[b]	1.23[b]	0.0290.000525
12718416	WFDC3	WAP four-disulfide core domain 3	1.00[a]	1.09[b]	-1.07[a]	0.0280.004584
12747918	XBP1	X-box binding protein 1	1.00[a]	1.08[a]	1.25[b]	0.0440.004947
12822034	XPNPEP1	X-prolyl aminopeptidase (aminopeptidase P) 1, soluble	1.00[a]	1.07[a]	1.25[b]	0.0400.000931

Table 5.5 (continued)

12903280	XPNPEP2	X-prolyl aminopeptidase (aminopeptidase P) 2, membrane	1.00[a]	-1.07[a]	-1.16[b]	0.0300.004889
12872296	YTHDC1	YTH domain containing 1	1.00[a]	-1.13[b]	-1.07[c]	0.0220.002949
12783927	YTHDF2	YTH domain family, member 2	1.00[a]	-1.05[b]	1.08[c]	0.0150.000152
12713839	YWHAB	tyrosine 3-monooxygenase	1.00[a]	1.04[b]	1.08[c]	0.0140.003731
12712681	ZC3H13	zinc finger CCCH-type containing 13	1.00[a]	-1.08[b]	1.02[a]	0.0140.000542
12704370	ZC3H6	zinc finger CCCH-type containing 6	1.00[a]	-1.28[b]	-1.31[b]	0.0570.004386
12773866	ZDBF2	zinc finger, DBF-type containing 2	1.00[a]	-1.93[b]	-1.20[a]	0.0920.000044
12891224	ZDHHC21	zinc finger, DHHC-type containing 21	1.00[a]	-1.28[b]	-1.09[a]	0.0530.004585
12720538	ZEB1	zinc finger E-box binding homeobox 1	1.00[a]	-1.15[b]	1.01[a]	0.0280.000738
12892852	ZFAND5	zinc finger, AN1-type domain 5	1.00[a]	-1.34[b]	-1.26[b]	0.0550.002168
12881969	ZFR2	zinc finger RNA binding protein 2	1.00[a]	1.06[a]	-1.07[b]	0.0220.003757
12907005	ZFX	zinc finger protein, X-linked	1.00[a]	-1.17[b]	-1.08[c]	0.0300.004194
12827158	ZMIZ1	zinc finger, MIZ-type containing 1	1.00[a]	1.18[b]	1.15[b]	0.0330.003687
12891595	ZNF189	zinc finger protein 189	1.00[a]	-1.20[b]	-1.06[a]	0.0340.001038
12753490	ZNF45	zinc finger protein 45	1.00[a]	-1.27[b]	-1.16[c]	0.0340.001503
12706050	ZNF638	zinc finger protein 638	1.00[a]	-1.07[b]	1.05[a]	0.0210.004503
12861600	ZNF641	zinc finger protein 641	1.00[a]	-1.21[b]	-1.01[a]	0.0440.003618
12840762	ZNF644	zinc finger protein 644	1.00[a]	-1.12[b]	-1.05[c]	0.0250.004517
12750954	ZNF773	zinc finger protein 773	1.00[a]	1.02[a]	1.16[b]	0.0310.002895

Table 5.5 (continued)

12702562	ZNF79	zinc finger protein 79	1.00[a]	-1.06[b]	1.11[c]	0.0230.000293

Se supplement treatments that contained 3 mg Se/day in the form of sodium selenite (ISe), Sel-Plex (OSe), or a 1:1 mix of ISe and OSe (MIX) were top-dressed onto enough of a common cottonseed hull/soybean hull/cracked corn-based diet

The abundance of gene transcripts is reported relative to the mean expression of the ISe group and are expressed as the fold change of non-transformed data

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

Means with different superscripted letters differ ($P < 0.1$)

The presented SEM values were pooled (averaged) from that of ISe (n = 6), OSe (n = 7), and MIX (n = 7) treatment group

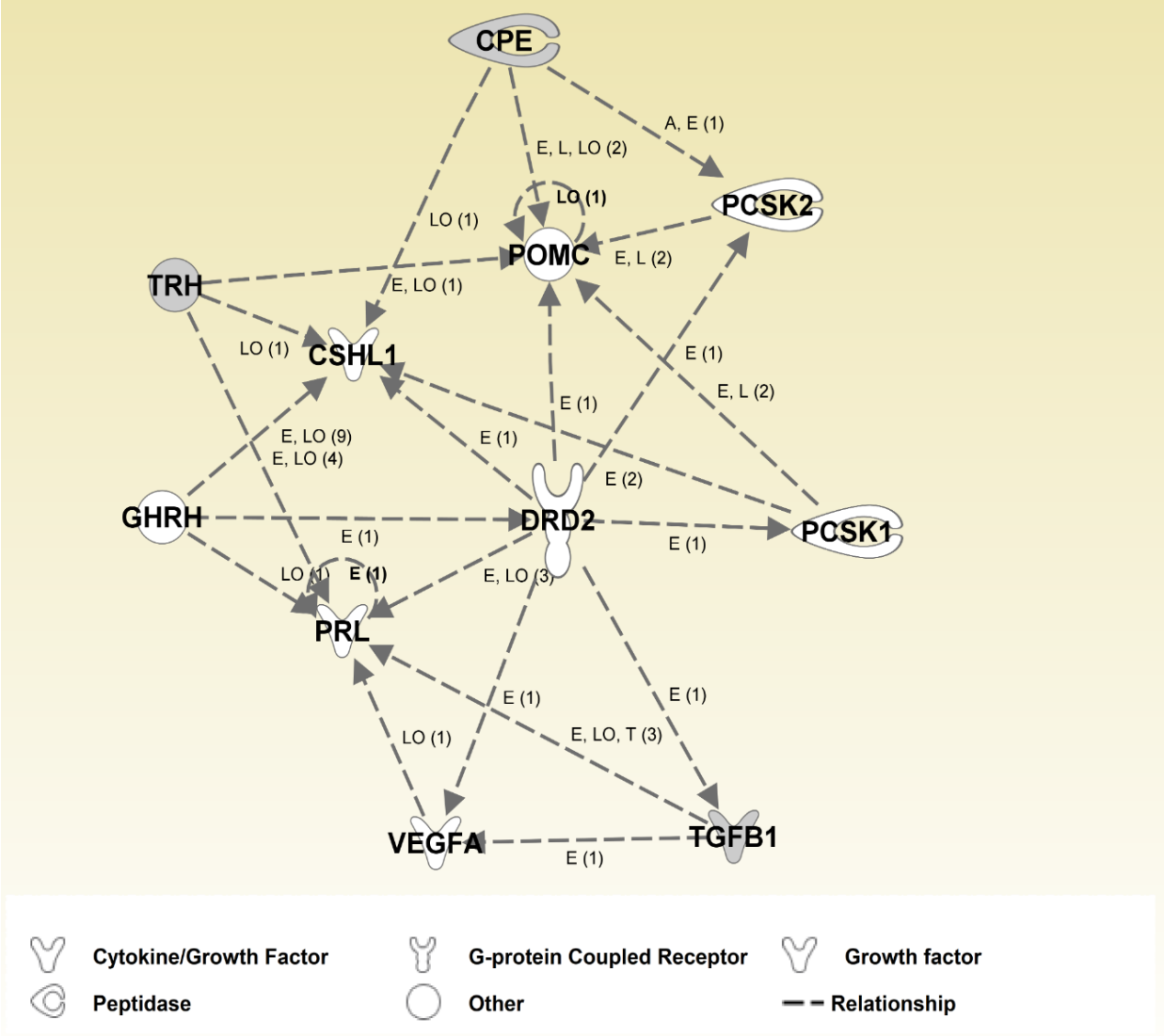

Figure 5.1. Canonical pathway network analysis. Shaded color indicates differentially expressed genes ($P < 0.005$). Non-shaded color indicates genes added from the Ingenuity Knowledge Base (Ingenuity Pathway, Ingenuity Systems, Inc., Redwood City, CA). Arrowheads symbolize action-on. Labels of interaction or relationship: A = Activation, E = Expression (includes metabolism or synthesis for chemicals), I = Inhibition, L = Molecular Cleavage, LO = Localization, T= Transcription. The number in parenthesis for each interaction indicates the number of published references in the Ingenuity Knowledge Base that support the particular interaction.

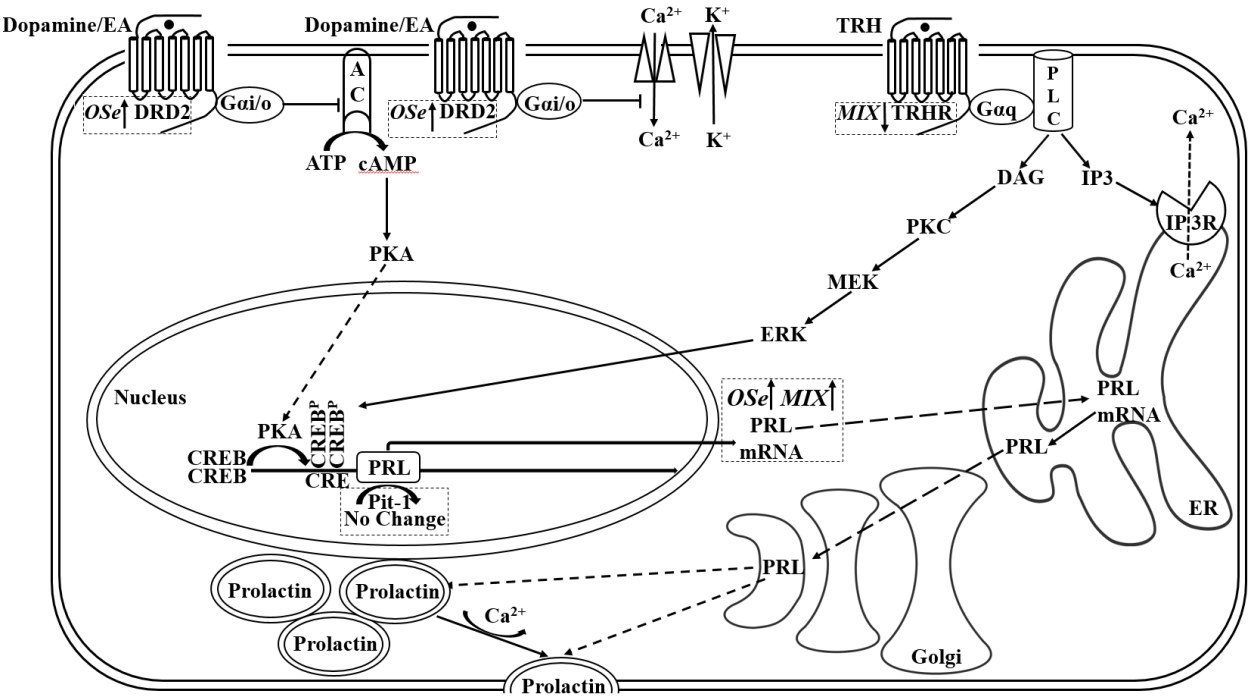

Figure 5.2. Mechanisms, and mRNA expression responses to Se form treatments, by which dopamine and TRH affect prolactin synthesis and release. AC, adenylyl cyclase; CRE, cAMP response element; CREB, cAMP response element binding protein; DAG, diacylglycerol; DRD2, dopamine receptor D2; EA, ergot alkaloid; ERK, extracellular signal-regulated kinase; IP3, inositol trisphosphate; ISe, sodium selenite; MEK, mitogen-activated protein kinase kinase; MIX, 1:1 mix of ISe and OSe; OSe, SEL-PLEX; Pit-1, pituitary-specific positive transcription factor 1; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PRL, prolactin; TRH, thyrotropin-releasing hormone; TRHR, thyrotropin-releasing hormone receptor. A line with arrowhead signifies interaction. A crosshead bar signifies inhibition. A dash line with arrowhead signifies transportation between cellular organelles. Adapted from Ben-Jonathan and Hnasko (2001) and Kanasaki et al. (2015). Sodium selenite (ISe), SEL-PLEX (OSe), or a 1:1 mix of ISe and OSe (MIX).

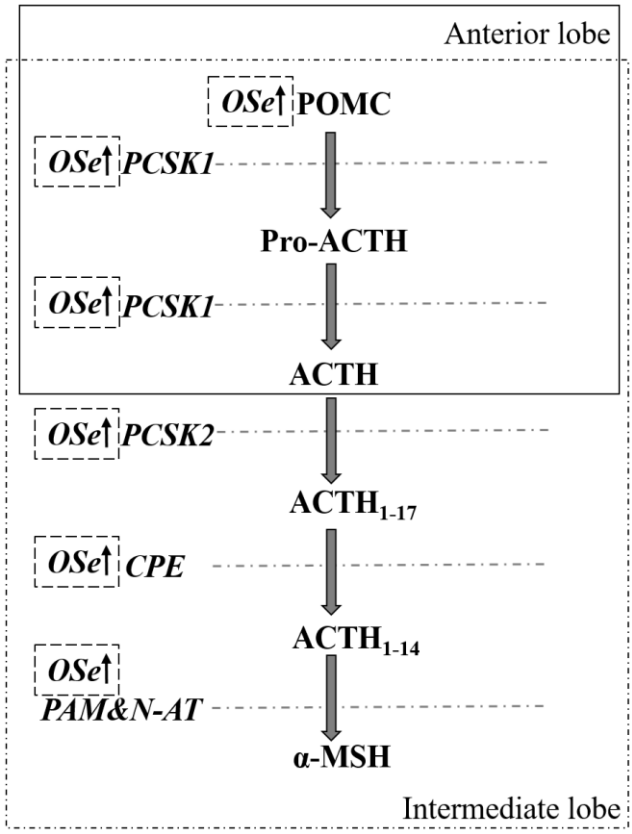

Figure 5.3. Regional biosynthesis of ACTH and α-MSH from POMC in the pituitary. CPE, carboxypeptidase E; MSH, melanocyte-stimulating hormone; N-AT, N-acetyltransferase; PAM, peptidylglycine α-amidating monooxygenase; PCSK: proprotein convertase subtilisin/kexin. Adapted from Getting (2006) and Cawley et al. (2016).

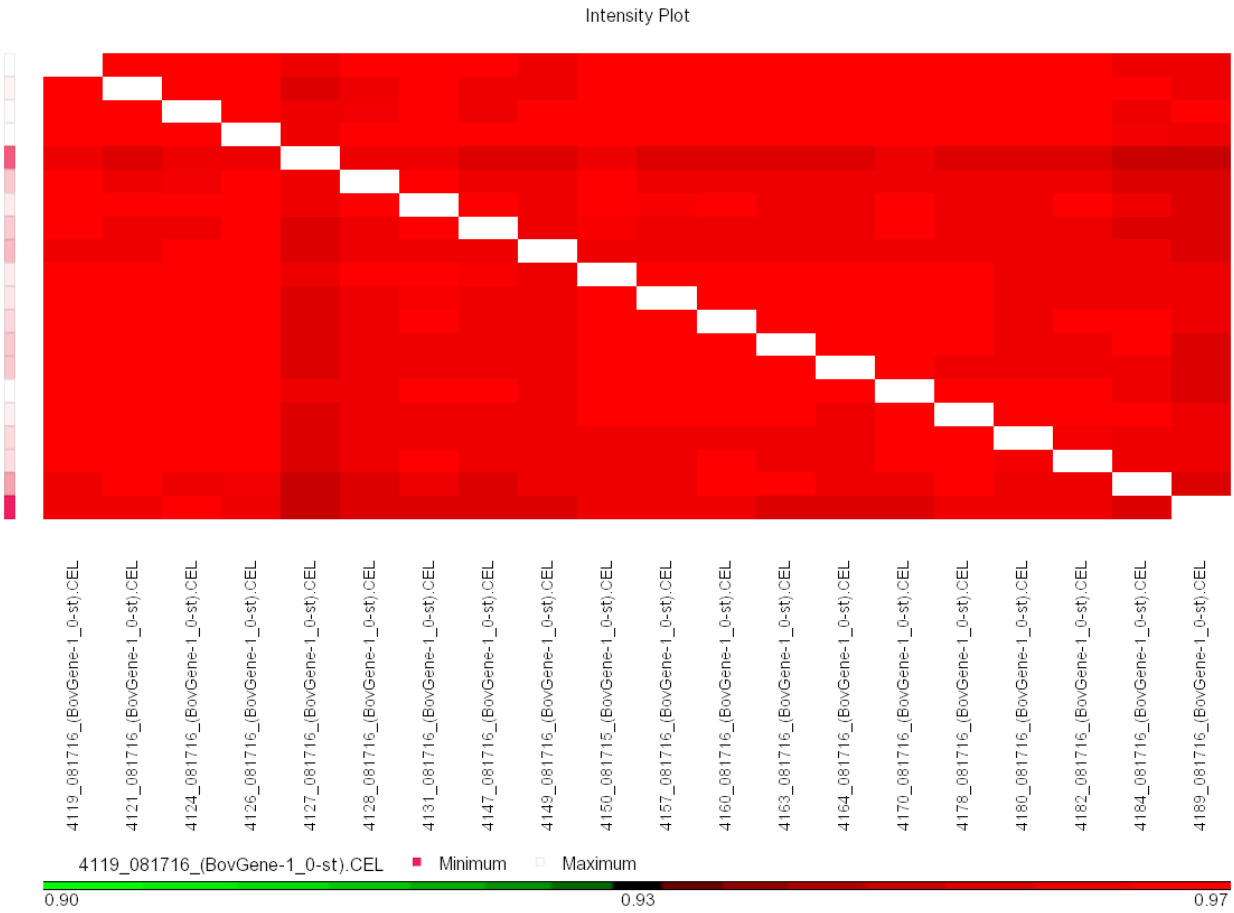

Figure 5.4. Correlation between microarray chips based on intensity values.

***RPS11*:**

AAGATGGCGGACATTCAGACAGAACGTGCGTACCAAAAGCAACCGACCATC
TTTCAAAATAAAAAGAGGGTCCTGCTTGGAGAAACTGGCAAAGAAAAGCTCC
CTCGATACTACAAGAACATTGGTCTGGGCTTCAAGACTCCAAAGGAGGCCAT
CGAGGGCACCTACATTGACAAGAAATGCCCTTTTACGGGTAATGTCTCATTCG
AGGGC

***TFRC*:**

CCAGGTTTAGTCTGGCTCGGCAAGTAGATGGTGATAACAGTCATGTGGAGAT
GAAATTAGCTGCAGATGAAGAAGAANAATGTTGACAGTAACATGAGGGGCA
ACCAAACCAGTATCGCAAAACCGAAAAGGTTAAATGGATATGTCTGCTACGG
GATCATTGCTGTAATCGTCTTTTTCTTGATTGGATTTATGATTGGCTACTTGGG
CTATTGTAGACGTGTGGAATCACAAGATTGTGGGAAAGAGGCAGGAACACA
GCCTTCGTGCCCAGGAGGAGACAGAAACTTTCGAATCAGAAGAGCAACTCCC
TGGAGTACCTCGCATATTCTGGGCAGACC

***UBC*:**

TAGGGGTGGGTTAGAGTTCAAGGTTTTTGTTCTACCAGATGTTTTAGTAGTAA
TCTGGAGGTAAGAAATGTCAAGAAAACATGGCCTTAATTAGAACTGTAGTGG
GTGAGTATAAATAAAAAATTTGGAGGTTGTAGTTAGAATTCTCCATATGTAC
ACTCATATGTAGATCTACTTATAAGCTACTGATTTTTAAAAGCACACGTTTGG
GAGTTGTGCTTAAGAGTGGGAAAGTTTCTGGAATACCAGCAGGGAGGT

***DRD2*:**

CGACCTTTCTCTGGGGCTTTGGGGCTCTGCGGCTGCGGGGCCAGTATCGAGGC
TCGGAGGCCTGGTTTTCACAGGCCATGCCGGAGCTGGTGGTGGGGAGGAGTG
GACAGTCACAGCCACCCAGGGCCCACACCTGAGAAGCCAGAGCTCTGGCCAC
GACCCCAGGCAGTGTCAAGCCTGGGAGACCCGCGTACACCCCAGGTCTGGAT
GGACCCCAGAGAAGCAGAAGCCCAA

***POU1F1 (Pit-1)*:**

AGCTGTGCATGGCTCTGAATTCAGTCAAACAACTATCTGCCGATTTGAAAACC
TGCAGCTCAGCTTCAAAAATGCATGCAAACTAAAAGCAATATTATCCAAATG
GCTGGAGGAAGCCGAGCAAGTAGGAGCTTTATACAATGAGAAAGTTGGTGC
AAATGAAAGAAAAAGGAAACGGAGAACAACAATCAGTATTGCTGCTAAAGA
CGCCCTGGAGAGACACTTTGGAGAACAGAATAAGCCTTCCTCTCAGGAGATC
CTGCGGATGGCTGAAGAACTAAACCTGGAGAAAGAAGTGGTGAGGGTTTGGT
TTTGTAACCGAAGGCAGAGAGAAAAACGGGTGAAGACAAGCCT

***PRL*:**

AGAACAAGCCCAACAGACCCACCATGAAGTCCTTATGAGCTTGATTCTTGGG
TTGCTGCGCTCCTGGAATGACCCTCTGTATCACCTAGTCACCGAGGTGCGGGG

TATGAAAGGAGCCCCAGATGCTATCCTATCGAGGGCCATAGAGATTGAGGAA
GAAAACAAACGACTTCTGGAAGGCATGGAGATGATATTTGGCCAGGTTATTC
CGAGCGCCAAAGAGACTGAGCCCTACCCTGTGTGGTCAGGACT

TRHR:

GCGATCTGTCACCCCATCAAAGCCCAGTTTCTCTGCACATTTTCCAGAGCCAA
AAAGATAATCATCTTTGTCTGGGCTTTCACATCCATTTACTGTATGCTCTGGTT
CTTCTTGCTGGATCTCAATATTAGCACCTATAAAGATGCTATTGTAGTGTCCT
GTGGCTACAAGATCTCCAGGAATTACTACTCACCTATTTACCTAATGGACTTT
GGTGTCTTTTATGTTGTGCCAATGATCCTAGCCACTGTCCTCTACGGAT

***VIP*:**

CTGGTTCAGCTGTAAGGGCAAGAGAACTCGTGAAGACTGTCGACTCCCAGGA
CTTCAACACCTGAGACAGCTCTCATAATCTCAACAGAAAGCTCTCAAAGAAC
ACTATTCGGCAAAGTCCTGCAATGGAAACAAGAAGTAAGCCCCAGCTTCTTG
TGTTCCTGACGCTGTTCAGCGTGCTCTTCTCCCAGACCTTGGCGTGGCCTCTTT
TTGGAGCACCTTCGGCTCTGAGGATGGGGGACAGAATACCATTTGAAGGAGC
GAATGAACCTGATCAAGTTTCGTTAAAAGCAGACACTGACATTTTACAAGAT
GCGCTGGCTGA

***GAL*:**

CACCGGTGAAGGAGAAGAGAGGCTGGACCCTGAACAGCGCTGGGTACCTTCT
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GCCGGCAAGCGGGAACTCGAGCCTGAAGACGAAGCCCGGCCAGGAAGCTTT
GACAGACCACTGGCGGAGAACAACGTCGTGCGCACGATAATCGAGTTTCTGA
CTTTCCTGCATCTCAAAGACGCC

VEGFA:

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CAAGATCCGCAGACGTGTAAATGTTCCTGCAAAAACACAGACTCGCGTTGCA
AGGCGAGGCAGCTTGAGTTAAACGAACGTACTTGCAGATGTGACAAGCCGAG
GCGGTGAGCCGGGCTGGAGGAAGGAGCCTCCCTCAGGGTTTCGGGAACCAG
ACG

TGFB1:

GCGGCCAGATTTGTCCAAGCTTCGGCTCGCCAGCCCCCCGAGCCAGGGGGAT
GTGCCACCCGGCCCGCTGCCCGAGGCCATACTGGCCCTTTACAACAGTACCC
GCGACCGGGTGGCCGGGGAAAGTGCCGAAACGGAGCCTGAGCCAGAGGCGG
ACTACTACGCCAAGGGAGGTCACCCGCGTGCTAAWGGTGGAATACGGCAAC
AAAATCTATGACAAAATGAAGTCTAGCCTCGCACAGC

GHRHR:

CTGTAACAGTCCTGTGTAAGGTTTCTGTGGCCACTTCCCATTTCGCTACCATG
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CTCCACATTGCCCAGCACAAGGAGGGTCTTCTGGTGGCTGGTTCTCGCTGCCT
GGGGGCTTCCTCTGCTCTTTACCGGCATGTGGGTGGGTTGCAAGTTGGCCTTC
GAAGAC

CSH2:

CCCCATGCTAAGCCCACAATTAACCCAACCAGTCCTGAGATGGTTAGTGATG
ATCCATCCCGTCAAAAGCTTCTTTGAGTTTTATAGCTCTTTAATGCATGTTTGG
GTGTAATGGGTCTCATCTGAAACAAAATAAACACAGATTCTGTAGAGATGTC
AAAATCTAAGAAGGAAATTTGTCAATGTCTTTATCTTCATTTAATAGAAAATC
AAAATAAAATCCATCCCTACCTCTGAGAGAAA

***L-PRLR*:**

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AAGGATTTGATGTTCATCTGCTGGAGAAGGG

***S-PRLR*:**

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GGCTATAGCATGGTGACCTGCATCCTCCCACCAGTTCCAGGGCCAAAAATAA
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***POMC*:**

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GCCGGTGTTCCCCGGCAACGGCGACGAGCAGCCGCTGACTGAGAACCCCCGG
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GCAG

***PCSK1*:**

TGATCGTGTGATATGGGCGGAACAACAGTATGAAAAAGAAAGAAGTAAACG
TTCAGTTCTAAGAGACTCAGCACTAGATCTCTTCAATGATCCGATGTGGAATC
AGCAGTGGTACTTGCAAGATACAAGGATGACTGCAACCCTGCCCAAGCTGGA
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PCSK2:

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TTTGGAGTCCGAAAGCTCCCCTTTGCTGAAGGCCTGTACCATTTTTATCACAA
TGGCCTGGCCAAGGCCAAGAGAAGACGCAGCCTACAGCATCAGCAGCAGCT
GGAGAGAGACCCCAGGGTAAAGAGG

CPE:

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CCCCATGTCGCAAGAATGATGATGACAGCAGCTTTGTAGAAGGAACGACCAA
TGGYGCTGCATGGTACAGCGTGCCTGGAGGAATGCAAGATTTCAATTACCTC
AGCAGCAACTGCTTTGAGATTACTGTGGAGCTTAGCTGTGAAAAGTTTCCACC
TGAAGAGACTTTGAAGAACTAYTGGGAGGATAACAAAAACTCCCTCATTAGC
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TAACCCAATTGCTAACGCCCCCTCTCTGTG

PAM:

ACCAACACCGTGTGGAAGTTCACCTCGACCGAAAAAATGGAACATCGATCAG
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TGAAACCAAAATGGAGAACAAGCCCGCCTCCTCAGAATTtGCAGAAGATACA
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ATTACAACCCTTCTGGTTATTCCTGTGGTTGTCCTGCTGGCCATTGCCTTATTT
ATTCGGTGGAAAAAATCAAGGGCCTTTGGAGATTCTGAAC

ATP5G1:

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TGATCAGGCCTGTGTCTGCCTCCTTCCTGAGTAGGCCAGAGATCCAATCTGTA
CAGCCTTCCTACAGCAGTGGCCCACTGCAGGTGGCCCGGCGGGAATTCCAGA
CCAGTGTTGTCTCCCGGGACATTGACACAGCGGCCAAGTTTATTGGCGCTGG
GGCTGCCACAGTTGGTGTGGCGGGTTCAGGGGCTGGTATTGGAACAGTGTTT
GGCAGCTTGATCATTGGCTATGCCAGGAACCCGTCTCTGAAGCAGCAGCTCT
CTCCTATGCCATTCTGGGCTTTGCCCTGTCTGAGGCTATGGGGCTCTT

COX7A2:

ACTGAGCCAAGATGCTACGGAATCTTCTGGCTCTCCGTCAGATTGCTAAGAG
GACCATAAGTACTTCTTCACGCAGGCAGTTTGAAAATAAGGTTCCAGAGAAA
CAAAAGCTGTTTCAGGAGGATAATGGAATTCCAGTGCATCTGAAGGGTGGGA
TAGCTGATGCCCTCCTGTATAGAGCCACCMTGATTCTTACAGTTGGTGGAAC
GGCATATGCCATGTATGAACTGGCTGTGGCTTCATTCCCAAGAAGCAGGATT
GACTTGAGTTTATCCTCCCAGCAATCAGTTGGGT

CYB5A:

AAGACTGCTTTGGTCCAGGGAGAAAGAAGCCACCACTCTTAACTTCAACTGA
CAACCCTTCACCTGAAAATAATCTGAATACACCTATTTTCCTTTCCTCCTACAT

TAGACACAAAACAAACCATAACTGTTCCATTCTTTGGACTATTGAACTTCTAA
AGTGTGCCTTCTTATTCACCAACTTTGTTTTGCTGTTCCATCACTACATCATTT
GCTTATTGTGGACATGATCTTTTAAAACATACCACCATGCTGAGCTGTC

FURIN:

GCATCGAGAAGAACCACCCAGACTTGGCAGGCAATTATGATCCTGGGGCCAG
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AATGACAACAGGCATGGCACACGGTGTGCAGGAGAGGTGGCTGCGGTGGCC
AACAATGGTGTCTGTGGCGTAGGCGTGGCCTACAATGCCCGAATTGGAGGGG
TGCGCATGCTGGATGGCGAAGTGACAGATGCCGTGGAG

GPX4:

GATCAAAGAGTTCGCCGCTGGCTATAACGTCAAATTCGATTTGTTCAGCAAG
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AGCCCAAGGGGAGAGGCATGCTGGGAAACGCCATCAAATGGAACTTCACCA
AGTTCCTCATTGACAAGAACGGCTGTGTGGTGAAGCGGTATGG

LRRK2:

AGGAATCCGATGCTTTGGCAAAACTTCGGAAAACCATCATCAATGAGAGCCT
TAATTTCAAGATCCGAGATCAGCCTGTTGTTGGGGCAGCTGATTCCAGACTGC
TACGTAGAACTTGAGAAAATCATTTTATCAGAGCGTAAAAATGTGCCAATTG
AATTTCCTGTAATTGACCGGAAACGATTATTACAACTTGTGAGAGAACATCA
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CAGGGGTCCTTCTTCATTTTCAAGACCCAGCATTGCAGT

NDUFA2:

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AATCCGCGAGTGCTCGGATGTGCAGCCCAAGCTCTGGGCCCGCTACGCATTT
GGCCAAGAGAAGAATGTCTCTCTGAACAATTTCAGTGCTGATCAGGTAACTA
GAGCCCTGGAGAACGT

PSENEN:

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TGCCCCAGACCCTCGGAAGACCGGCCCCAGCGCAACTATGAACTTGGAGCGG
GTGTCCAACGAAGAGAAGTTGAACCTCTGCCGGAAGTACTACCTGGGTGGGT
TTGCCTTCCTGCCTTTTCTCTGGTTGGTCAACATTTTCTGGTTCTTCCGAGAGG
CCTTCATTGTCCCGGCATACACGGAGCAGAGCCAAATCAA

SDHB:

AGAGACGACTTCACGGAGGAGCGCCTGGCCAAGCTGCAGGACMCCTTCTCTC
TCTACCGCTGCCACACCATCATGAACTGCACGCAGACCTGCCCCAAGGGGCT
GAATCCTGGGAAAGCTATTGCTGAAATCAAGAAGATGATGGCAACCTATAAA

GAGAAGCAGGCTTCTGCTTAACTGCCGTGCTCAGCCTGACTGGAGCCGGCTC
AGAATGCAGTTCACCCCTGAGTTCCTTCAG

CAT:

CTATCCTGACACTCACCGCCACCGCCTGGGACCCAACTATCTCCAGATACCTG
TGAACTGTCCCTACCGTGCTCGAGTGGCCAACTACCAGCGTGACGGCCCCAT
GTGCATGATGGACAATCAGGGTGGGGCTCCAAATTACTACCCCAATAGCTTT
AGTGCTCCCGAGCATCAGCCTTCTGCCCTGGAACACAGGACCCACTTCTCTGG
GGATGTACAGCGCTCAACAGTGCCAACGATGACAATGTCACTCAGGTGCGGA
CTTTC

SOD1:

TTGGAGACCTGGGCAATGTGACAGCTGACAAAAACGGTGTTGCCATCGTGGA
TATTGTAGATCCTCTGATTTCACTCTCAGGAGAATATTCCATCATTGGCCGCA
CGATGGTGGTCCATGAAAAACCAGATGACTTGGGCAGAGGTGGAAATGAAG
AAAGTACAAAGACTGGAAACGCTGGAAGCCGTTTGGCCTGTGGTGTAA

Figure 5.5. The sequences of the real-time RT-PCR products (5’ to 3’ orientation). Within a sequence, underlined nucleotides indicate the forward and reverse primer positions.

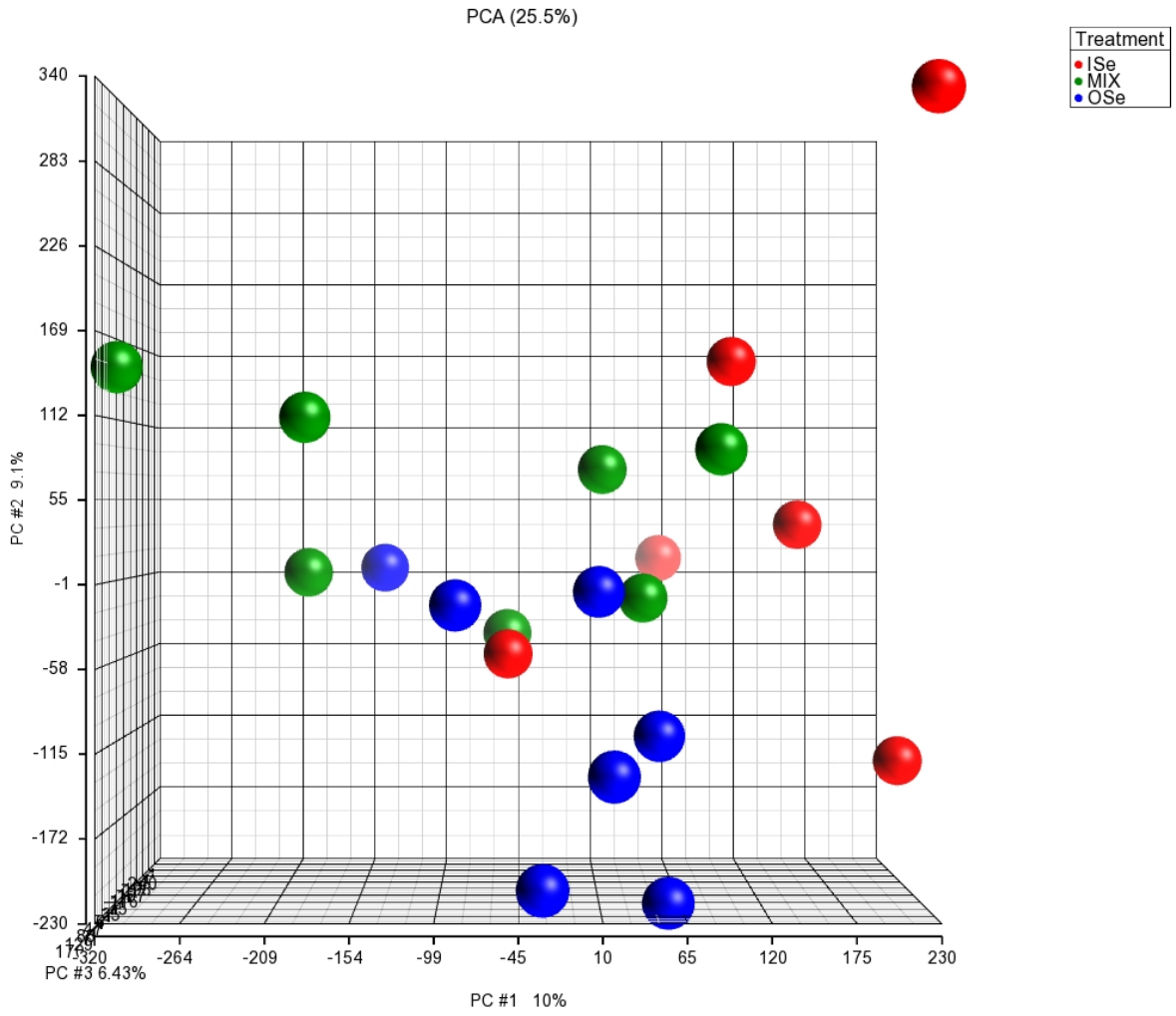


Figure 5.6. Principle component analysis of microarray transcriptome analysis of 20 pituitary samples from steers grazing endophyte-infected tall fescue and supplemented with 3 mg Se/d in vitamin-mineral mixes as either sodium selenite (ISe, n = 6, red dots), SEL-PLEX (OSe, n = 7, blue dots), or a 1:1 mix of ISe and OSe (MIX, n = 7, green dots). The red, blue, and green dots represent linear combinations of the relative expression data, including expression values and variances, of the 26,773 gene transcripts in each Bovine Chip.

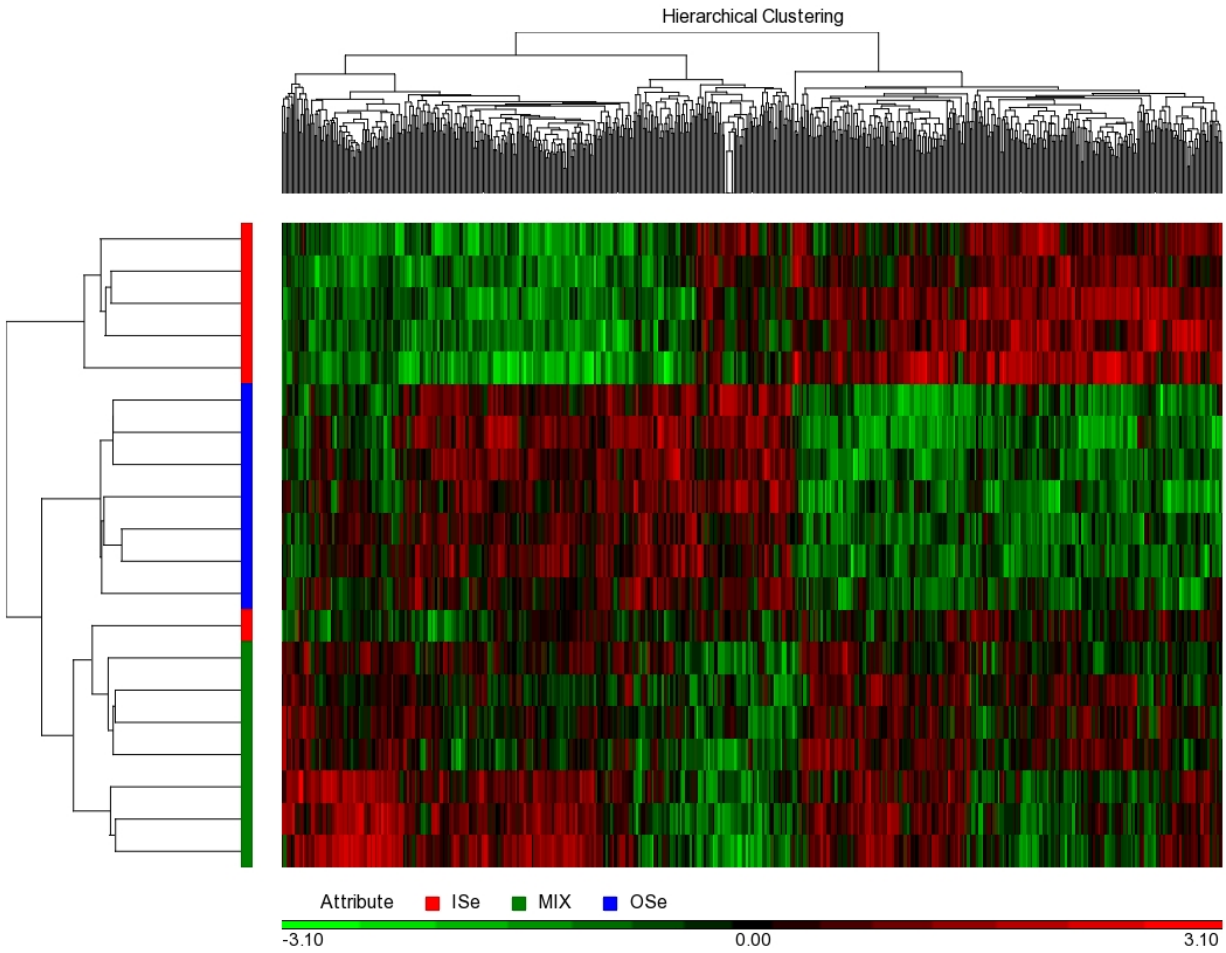


Figure 5.7. Hierarchical cluster analysis of the 542 “focus” genes selected as differentially expressed (ANOVA P-values of < 0.005 and false discovery rates of ≤ 18.8%) by the pituitary of steers grazing endophyte-infected tall fescue and supplemented with 3 mg Se/d in vitamin-mineral mixes as either sodium selenite (ISe, n = 6), SEL-PLEX (OSe, n = 7), or a 1:1 mix of ISe and OSe (MIX, n = 7). As indicated by the legend color box, black color in the middle represents the mean value, 0; red color represents gene expression levels above the mean expression; and blue color denotes expression below the mean. The intensity of the color reflects the relative intensity of the fold change.

CHAPTER 6. Selenoprotein Gene Expression Profiles in the Pituitary and Liver of Growing Steers Grazing Endophyte-Infected Tall Fescue Are Sensitive to Different Forms of Supplemental Selenium

6.1 Abstract

The goal of this study was to test the hypothesis that the gene expression profiles of 25 selenoproteins in the pituitaries and livers of growing beef steers commonly grazing an endophyte-infected tall fescue (HE) pasture would differ after consuming different forms of selenium (sodium selenite (ISe), SEL-PLEX (OSe), vs. a 1:1 blend (MIX) of ISe and OSe) in a basal vitamin-mineral (VM) mix. Predominately-Angus steers (BW = 183 ± 34 kg) were randomly selected from herds of fall-calving cows grazing HE pasture and consuming VM mixes that contained 35 ppm Se as ISe, OSe, or MIX forms. Steers were weaned, depleted of Se for 98 d, and subjected to summer-long common grazing of a 10.1 ha HE pasture containing 0.51 ppm ergot alkaloids. Steers were assigned (n = 8 per treatment) to the same Se-form treatments on which they were raised. Selenium treatments were administered by daily top-dressing 85 g of VM mix onto 0.23 kg soyhulls, using in-pasture Calan gates. As previously reported, whole blood Se was higher for OSe (11%) and MIX (7.5%) steers vs. ISe. Pituitaries and livers were collected at slaughter and changes in selenoprotein mRNA expression patterns determined by microarray and real-time reverse-transcription PCR analyses, respectively. The effects of Se treatment on relative gene expression were subjected to one-way ANOVA. In the pituitary, mRNA contents of 7 selenoproteins and two selenoprotein P receptors differed among Se treatments, whereas two selenoproteins were affected in liver. OSe steers may have a greater capacity to manage against oxidative damage, maintain cellular redox

balance, and have a better quality control of protein-folding in their pituitaries than ISe steers. In contrast, the liver tissue of MIX steers may have a greater capacity for redox signaling and a greater ability to manage oxidative stress than ISe steers. We conclude that the form of Se in vitamin-mineral mixes alters the selenoprotein transcriptome of both the pituitary and liver of growing steers consuming HE forage, in an ostensibly positive manner.

KEYWORDS: cattle, fescue toxicosis, liver, pituitary, selenium supplementation, selenoprotein profile

6.2 Introduction

Selenium (Se) is an important trace element for many southeastern United States cattle producers due to low-Se soils (Dargatz and Ross, 1996). Selenium deficiency causes a variety of negative effects on beef cattle production including increased incidence of early embryonic death, retained placentas, cystic ovaries and weak heat periods (Corah, 1996), and reduced growth rate and immune responses (Cerny et al., 2016). Se-poor soils result in Se-deficient forages necessitating the need to provide supplemental Se (Dargatz and Ross, 1996). Inorganic Se (ISe, sodium selenite) is the most common form of Se supplemented in cattle diets, whereas organic forms of Se (OSe) derived from specially cultivated *Saccharomyces cerevisiae* also are available and approved for beef cattle production (Juniper et al., 2008).

The other challenge faced by many southeastern United States cattle producers is fescue toxicosis, which results from consumption of ergot alkaloids found in *Epichloe coenophialum*-infected tall fescue (*Lolium arundinaceum*) pastures and is a clinical condition consisting of impaired metabolic, vascular, growth, and reproductive processes in cattle (Strickland et al., 2011). Serendipitously, it was found that expression of several genes downregulated in the liver (Liao et al., 2015) and pituitary (Li et al., 2017) of steers grazing high vs. low endophyte-infected forages were upregulated in cattle by consumption of a 1:1 blend of ISe:OSe (MIX) in vitamin-mineral (VM) mixes (Matthews and Bridges, 2014; Matthews et al., 2014).

It is widely believed that Se exerts its biological functions through selenoproteins, most of which are redox enzymes. There are 25 identified mammalian selenoprotein genes (Kryukov et al., 2003). The goal of the present study was to test the hypothesis that

the form of supplemental Se would alter selenoprotein transcriptome profiles in pituitaries and livers of growing steers subjected to summer-long grazing of endophyte-infected pasture.

6.3 Materials and methods

All experimental procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee.

6.3.1 Animals model

The animal management regimen and model for steers that yielded the pituitary and liver tissues of the present experiment have been reported (Jia et al., 2018). Briefly, twenty-four predominantly Angus beef steers (BW, 182.6 ± 33.9 kg; age, 165.5 ± 14.2 d) were randomly selected from 3 Se phenotypic herds (8 steers/herd), which were managed under a typical forage-based (predominately endophyte-infected tall fescue), fall-calving, cow-calf production regimen. The 3 Se phenotypic herds had free access to VM premixes (UK Beef IRM Cow-Calf Mineral, Burkmann Feeds, Danville, KY) containing 35 ppm of inorganic Se (ISe, sodium selenite, Prince Se Concentrate; Prince Agri Products, Inc., Quincy, IL, USA), organic Se (OSe, SEL-PLEX, Alltech Inc., Nicholasville, KY), and 1:1 mix of ISe:OSe (MIX). After adapted to consuming VM premixes from in-pasture Calan gate feeders, twenty-four steers with 3 Se phenotypes (n = 8) started (d 0) summer-long grazing (86 d) of a 10.1-ha predominately endophyte-infected tall fescue-mixed pasture (0.51 μg/g total ergot alkaloids) (Jia et al., 2018). The 3 Se form treatments were administered using in-pasture Calan gate feeders to steers with the same Se phenotypes. All 3 Se form treatments contained a common basal VM premix with added 35 ppm Se as either ISe, OSe, or MIX. During this 86-d period, two ISe steers

were removed from the trial due to a bad hoof and failure to consume their mineral treatment. After the common 86-d grazing period on pastures, steers were slaughtered in the University of Kentucky Meat Laboratory (Lexington, KY) over a 26-d period (from day 93 to 119, 8 slaughter d, 1 steer from each treatment/d). Throughout the slaughter period, steers continued to maintain their respective Se treatment. Details of the slaughter period and process have been reported (Jia et al., 2018).

6.3.2 Sample collection and RNA preparation

Steers were stunned by captive bolt pistol and exsanguinated. Within 15 to 20 minutes of death, the whole pituitary and liver samples collected from mid-lower right lobe were collected from each animal, placed in a foil pack, flash-frozen in liquid nitrogen, and stored at -80°C. Two pituitary glands (1 OSe and 1 MIX) were not used because of tissue damage incurred during the collection process. As a result, six pituitaries (n = 6) and six liver samples (n = 6) for ISe, and seven pituitaries (n = 7) and eight liver samples (n = 8) for both OSe and MIX treatment groups were subjected to RNA analyses.

Total RNA was extracted from the whole frozen pituitary and liver tissue using TRIzol Reagent (Invitrogen Corporation, Carlsbad, CA) following the manufacturer's instructions. The RNA concentrations were determined using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE), which revealed that all samples had an average concentration of 706 ng/μL for pituitary and 1.05 μg/μL for liver samples, and were of high purity with 260:280 nm absorbance ratios ranging from 1.85 to 2.05 and 260:230 nm absorbance ratios ranging from 1.89 to 2.50. The integrity of total RNA was examined by gel electrophoresis using an Agilent 2100 Bioanalyzer System

(Agilent Technologies, Santa Clara, CA) at the University of Kentucky Microarray Core Facility. All RNA samples had 28S:18S rRNA absorbance ratios greater than 1.8 and RNA integrity numbers greater than 8.7.

6.3.3 Microarray analysis

The GeneChip Bovine Gene 1.0 ST Array (Affymetrix, Inc., Santa Clara, CA) was used to investigate the effect of Se treatment on bovine pituitary and liver gene expression profiles. Microarray analysis was conducted according to the manufacturer's standard protocol at the University of Kentucky Microarray Core Facility. Briefly, 3 μg of RNA for each sample was first reverse-transcribed (RT) to cDNA and then from cDNA (double-stranded) to complementary RNA (cRNA; single-stranded), which was then labeled with biotin. The biotinylated cRNAs were further fragmented and used as probes to hybridize the gene chips in the GeneChip Hybridization Oven 640 (Affymetrix), using 1 chip per RNA sample. After hybridization, the chips were washed and stained on a GeneChip Fluidics Station 450 (Affymetrix). The reaction image and signals were read with a GeneChip Scanner (GCS 3000, 7G; Affymetrix), and data were collected using the Command Console. The raw expression intensity values from the Command Console (i.e., 20 *.cel files from the raw methylation measurements) were imported into Partek Genomics Suite software (PGS 7.17.0918; 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 chips and median polish summarization of multiple probes for each probe set.

All the GeneChip transcripts were annotated using the NetAffx annotation database for gene expression on Bovine GeneChip Array ST 1.0, provided by the manufacturer (http://www.affymetrix.com/analysis/index.affx, last accessed in January 2018, annotation file last updated in May 2016). Quality control of the microarray hybridization and data presentation was performed by MA plot on all the gene expression values and by box plot on the control probe sets on the Affymetrix chips (data not shown). To assess treatment effects (ISe vs. OSe vs. MIX) on the relative expression of the pituitary and liver gene transcripts, qualified microarray data were subjected to one-way ANOVA using the same PGS software.

All the microarray *.cel files collected by Command Console plus the GC Robust Multichip Averaging-corrected data processed by PGS software of this manuscript have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus (**GEO;** http://www.ncbi.nlm.nih.gov/geo/) [released May 25, 2018]), are minimum information about a microarray experiment (**MIAME**) compliant (Brazma et al., 2001), and are accessible through GEO series accession number GSE114893 (pituitary) and GSE115802 (liver).

6.3.4 Real-time RT-PCR analysis

Primer sets for genes selected for real-time reverse transcription (RT) PCR analysis (Table 6.3) were designed using the NCBI Pick Primers online program against RefSeq sequences. Real-time RT-PCR was performed using an Eppendorf Mastercycler ep *realplex*2 system (Eppendorf, Hamburg, Germany) with iQ SYBR Green Supermix (Bio-RAD, Hercules, CA), as described (Bridges et al., 2012). Briefly, cDNA was synthesized using the SuperScript III 1st Strand Synthesis System (Invitrogen), with 1 μg

of RNA used for each reverse transcription reaction. Real-time RT-PCR was performed with a total volume of 25 μL per reaction, with each reaction containing 5 μL of cDNA, 1 μL of a 10 μM stock of each primer (forward and reverse), 12.5 μL of 2× SYBR Green PCR Master Mix, and 5.5 μL of nuclease-free water. Gene expression was analyzed by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

The resulting real-time RT-PCR products were purified using a PureLink Quick Gel Extraction Kit (Invitrogen) and sequenced at Eurofins Scientific (Eurofins, Louisville, KY). Sequences were compared with the corresponding RefSeq mRNA sequences used as the templates for primer set design. The sequences of the primers and the resulting sequence-validated real-time RT-PCR reaction amplicons for selected DEG and the endogenous control genes (RPS11, TFRC, and UBC for pituitary; GAPDH, PCK2, and SDHA for liver) are presented in Table 6.3 and Figure 6.3, respectively. All sequenced amplicons had at least 98% identity with their template sequences. Three constitutively expressed housekeeping genes for pituitary (RPS11, TFRC, and UBC; P = 0.59, 0.51, 0.66; respectively) and liver (GAPDH, PCK2, and SDHA; P = 0.60, 0.78, 0.75; respectively) were used and their CT values were not affected by Se-form treatments. Thus, the geometric mean expression of 3 housekeeping genes was used to normalize the relative quantities of the selenoprotein mRNA expression, and all RT-PCR reactions were conducted in triplicate.

6.3.5 Statistical analysis

Steers were the experimental units. To test for Se treatment effects on the relative expression of the selenoprotein gene transcripts analyzed by microarray hybridization, data were subjected to one-way ANOVA using the PGS software as described in the

"Microarray Analysis" section above. To determine the effects of treatment on the relative expression levels of selenoprotein gene analyzed by real-time RT-PCR, data were subjected to one-way ANOVA using the GLM procedure of the SAS statistical software package (version 9.4; SAS Inst., Inc., Cary, NC), with the Se treatment as the fixed effect. For these data, significance was declared when $P \leq 0.05$, and a tendency to differ was declared when $0.10 > P > 0.05$. When $P < 0.10$, means were separated using Fisher's LSD procedure. Principal component analysis (PCA) was performed using JMP Pro software (version 14; SAS Inst. Inc.). As appropriate, Se concentration, glutathione (GSH) content, glutamine synthetase (GS) activity, and relative mRNA abundance of selenoproteins in liver were used as input variables for PCA.

6.4 Results

6.4.1 Microarray and real-time RT-PCR analyses of selenoprotein mRNA

In the pituitary, real-time RT-PCR analysis corroborated the microarray analysis that the relative expression of GPX3, LRP2, LRP8, SELENON, SEPHS2, TXNRD1 differed ($P < 0.05$) among Se treatment steers (Table 6.1). In addition, both techniques showed that the relative expressions of DIO1, DIO2, DIO3, GPX1, GPX2, SELENOI, SELENOK, SELENOP, SELENOT, SELENOV, SELENOW, SEPHS1, and TXNRD3 were not affected by Se treatment (Table 6.1). The relative expression of GPX4, MSRB1, and TXNRD2 differed ($P < 0.05$) based on microarray analysis, whereas RT-PCR results showed their tendency to differ ($P < 0.1$). In contrast, RT-PCR showed SELENOM mRNA affected by Se treatment ($P < 0.05$), but according to microarray, SELENOM mRNA had the tendency to change ($P < 0.1$). There was inconsistency between microarray and RT-PCR results for 4 genes. The relative expression of GPX6,

SELENOF, and SELENOS differed or tended to differ by RT-PCR analysis, but not by microarray analysis. In addition, RT-PCR found that SELENOO was affected by Se treatment, but this gene is not included in the microarray chip design. With regard to fold-changes, the direction of Se treatment-induced change was the same between microarray and RT-PCR analyses whereas the magnitude of the determined fold-changes typically was greater by RT-PCR analysis.

In liver, real-time RT-PCR analysis corroborated the microarray analysis finding that the relative expression of SELENOT differed ($P < 0.05$) and that of GPX4 tended to differ ($P < 0.1$) among Se treatment steers (Table 6.2). In addition, both techniques showed that the relative expression of DIO1, GPX1, GPX2, GPX3, LRP2, LRP8, MSRB1, SELENOF, SELENOI, SELENOK, SELENON, SELENOV, SELENOW, SEPHS1, and TXNRD3 was not affected by Se treatment (Table 6.2). The relative expressions of SELENOS and TXNRD1 tended to differ ($P < 0.1$) based on microarray analysis, whereas RT-PCR results showed that they do differ ($P < 0.05$) among Se treatment steers. As for the pituitary, inconsistencies existed between results of microarray and RT-PCR analyses. The relative expressions of DIO3, SELENOP, SEPHS2, and TXNRD2 differed or tended to differ by RT-PCR analysis, but not by microarray. Whereas microarray analysis showed that relative expressions of DIO2, GPX6, and SELENOM were affected by Se treatment ($P < 0.1$), but RT-PCR analysis did not. RT-PCR also found that SELENOO was affected by Se treatment but, as noted above, this gene is lacking in the GeneChip Bovine Gene 1.0 ST Array.

6.4.2 Principal component analysis

Principal component analysis of the potential correlation between mRNA contents of selenoproteins and other analytes (Se concentration, glutathione content, and glutamine synthetase activity) in liver indicated that PC1 and PC2 explained 37.7% of the variation (Figure 6.1 and 6.2). All steers with ISe supplementation segregated within the negative quadrants of PC2, whereas three steers from both OSe and MIX groups segregated into the negative quadrants of PC2 (Figure 6.1). Relative mRNA expression of GPX2, SELENOP and SELENOV, GSH content, and Se concentration clustered in the PC1-positive and PC2-negative quadrant (Figure 6.2). They were either weakly or not correlated with PC1 and PC2.

6.5 Discussion

It is widely accepted that Se exerts its biological function mainly through selenoproteins. However, most studies focusing on effect of supplemental Se on selenoprotein expression or activity used the Se deficiency vs. Se supplementation model. Using a Se-adequate model, our laboratory has shown that forms of supplemental dietary Se affected the expression of certain genes in the livers of maturing beef heifers (Matthews et al., 2014), neonatal testes (Cerny et al., 2016), and pituitaries of growing beef steers (Chapter 5). We also have shown that consumption of HE forage decreases the expression of at least 7 selenoproteins by the liver or pituitaries of growing steers (Matthews and Bridges, 2014; Liao et al., 2015; Li et al., 2017) that are upregulated by MIX or OSe forms of supplemental Se. However, to our knowledge, the potential effects of forms of supplemental Se on the selenoprotein transcriptome of the pituitary and liver of cattle grazing HE forages has not been reported. To obtain this information and

elaborate the findings of Chapter 5, we conducted microarray and RT-PCR analyses of 25 selenoprotein genes in both pituitary and liver tissues collected from previously described (Chapter 5) beef steers, which had consumed VM mixes that contained 35 ppm Se as ISe, OSe, or MIX forms while subjected to summer-long grazing of high endophyte-infected tall fescue (Jia et al., 2018). That OSe and MIX steers had 11% and 7.5% more whole blood Se than ISe steers, respectively, and did not differ from each other, as expected, validated the successful administration of the Se treatments.

In pituitary tissue, the expression of 5 selenoproteins (GPX3, GPX4, MSRB1, SELENON, TXNRD1) were increased ($P \leq 0.05$) and one (SEPHS2) decreased ($P \leq 0.04$) by Se treatments, based on both microarray and RT-PCR analyses. In addition, one selenoprotein (SELENOO), solely evaluated by RT-PCR, was increased ($P = 0.03$). Also, the expression of the two receptors for selenoprotein P (LPR2, LPR8) was affected ($P \leq 0.04$) in the pituitary. In the liver, the expression of only two selenoproteins (SELENOO, SELENOT) was affected (increased, $P \leq 0.04$), according to both microarray and RT-PCR, or only RT-PCR. Inconsistencies existed between the results of the microarray and RT-PCR analyses, especially in liver. Possible explanations for these inconsistencies include differences in the sites and number of nucleotides measured for a given transcript, gene normalization procedures, and inherent technique-specific pitfalls (Bustin, 2002; Chuaqui et al., 2002; Morey et al., 2006). Because RT-PCR often is considered the more accurate technique for quantification of gene expression, due to its greater sensitivity and specificity (Schmittgen et al., 2008; Derveaux et al., 2010), when there was a conflict between microarray and RT-PCR results, we used the RT-PCR finding as the definitive finding.

Glutathione peroxidases

The family of glutathione peroxidases (GPx) catalyze the reduction of hydrogen peroxide (H_2O_2) or hydroperoxides to water (at the expense of oxidizing 2 GSH to GSSG) to maintain cellular redox homeostasis (Margis et al., 2008). The five known mammalian GPx are all selenoproteins. Cytosolic glutathione peroxidase (GPx1) is the first identified mammalian selenoprotein, which scavenges toxic H_2O_2 and soluble organic peroxides (Rotruck et al., 1973). GPx1 is expressed in all cell types and is most abundant in the liver and kidney. Functionally, GPx2 highly resembles GPx1. However, unlike GPx1 which is a ubiquitously expressed, GPx2 is expressed mainly in the epithelium of gastrointestinal tract and somewhat in liver (Arthur, 2001). Glutathione peroxidase 3 and GPx4 are expressed by a wide range of cell types and tissues. GPx3 is the only extracellular GPx isoform and is secreted mainly by the kidney. Importantly, as the concentration of plasma GSH is fairly low, GPx3 can utilize thioredoxin or glutaredoxin as reductants in addition to GSH (Björnstedt et al., 1994). Unlike GPx1, GPx2, and GPx3, which are all homotetramers, GPx4 is ubiquitously present in the cytosol as a monomer, which enables its unique reduction function of complex phospholipid hydroperoxides in membranes (Herbette et al., 2007; Labunskyy et al., 2014). Glutathione peroxidase 4 also uses protein thiols as alternate substrates besides GSH (Ursini et al., 1999). Glutathione peroxidase 6 is the last identified Sec-containing glutathione peroxidase, and its mRNA has been reported to be only found in embryos and olfactory epithelium (Kryukov et al., 2003).

In the pituitary, RT-PCR analysis found that OSe steers had more GPx3 (180%) and GPx4 (51%) mRNA than ISe and MIX steers, whereas MIX steers had less GPx6

(54%) than ISe steers. In the liver, GPx4 mRNA content was greater (55%) in MIX vs. ISe steers (Table 6.2). Collectively, these findings suggest that OSe and MIX steers had a greater capacity to prevent oxidative damage and to maintain cellular redox balance better in their pituitaries and livers, respectively.

In liver, PCA indicated that GSH content was closely associated with Se concentration (Figure 6.2). The mRNA content of GPx2 was closely associated with hepatic GSH content in the PC1-positive and PC2-negative quadrant. In addition, the mRNA content of GPx1 (the most abundant mammalian selenoprotein), also existed in that quadrant and closely associated with GPx2. In contrast, GPx4 (another abundant liver glutathione reductase) and other GSH-interacting selenoproteins TXNRD2 and SELENOW were not closely associated with hepatic GSH content (Figure 6.2). Hence, it is possible that hepatic Se concentration affected hepatic GSH content by regulating the expression of GPx1 and GPx2. Interestingly, PCA also indicated that glutamine synthetase activity was present in the opposite quadrant of GSH. This finding seems to support a previous suggestion that the GSH synthesis enzyme γ-glutamylcysteine ligase and glutamine synthetase may directly compete for hepatic Glu (Huang et al., 2018).

Thioredoxin reductases

Thioredoxins (TXNRD) are a class of small, ubiquitously-expressed redox proteins present in all living cells, protecting cells from oxidative stress and facilitating redox signaling by catalyzing the dithiol-disulfide exchange reactions of other proteins. Oxidized thioredoxins need to be reduced to exert their functions again, and the NADPH-dependent reduction is catalyzed by the flavoenzyme thioredoxin reductases (Arnér, 2009). There are three known thioredoxin reductases in mammals, all of which are

selenoproteins (Labunskyy et al., 2014). Thioredoxin reductase 1 is primarily present in cytosol, and is the major protein disulfide reductase in mammalian cells (Arnér and Holmgren, 2000). Thioredoxin reductase 2 is localized to mitochondria and expressed in various cell types (Rundlöf et al., 2001; Lillig and Holmgren, 2007), and thioredoxin reductase 3 exists primarily in testes (Arnér, 2009).

In the pituitary, the abundance of TXNRD1 and TXNRD2 mRNA was greater in MIX steers than both ISe and OSe steers. (Table 6.1). In liver, that MIX and OSe steers had more TXNRD1 mRNA than ISe steers and that ISe, and MIX steers had more TXNRD2 mRNA than OSe steers (Table 6.2), suggests that MIX steers may have had better thioredoxin-thioredoxin reductase system in both pituitary and liver tissues.

Selenoprotein P and its receptors

Unlike most other selenoproteins which usually contain one Sec residue, selenoprotein P (SelP) contains 9-12 Sec residues per protein molecule in mammals (Read et al., 1990). Bovine SelP contains 12 Sec residues (Mostert, 2000). This unique property enables SelP to account for approximately 50% of the total Se in plasma (Burk and Hill, 2005). It has been suggested that the main role of SelP is in selenium transport, delivery, and storage (Burk et al., 1991; Saito and Takahashi, 2002). Selenoprotein P is mainly synthesized in liver, although its mRNA can be found in all tissues (Labunskyy et al., 2014). There are two endocytic receptors for SelP from low-density lipoprotein receptor family facilitating SelP entering into extrahepatic cells in a tissue-specific manner, the major receptor apolipoprotein E receptor-2 (apoER2) (Olson et al., 2007) and the minor receptor megalin (Olson et al., 2008). ApoER2 is expressed highly in testis, bone marrow, placenta, brain, and muscle; moderately in other tissues like thymus and

spleen; and minimally in liver and kidney (Burk and Hill, 2015). It is suggested the tissue hierarchy for Se in largely determined by SelP binding to apoER2 (Burk and Hill, 2015). The other SelP receptor megalin plays an essential role for uptake of plasma SelP in kidney, and the uptake is proposed to provide Se for GPx3 synthesis in kidney proximal tubules (Avissar et al., 1994).

The SELENOP mRNA content in the pituitary was not affected by Se treatment. However, MIX steers had more apoER2 mRNA and less megalin mRNA than ISe steers, suggesting that MIX steers may have had more Se uptake in their pituitaries than ISe steers. In the liver, OSe (19%) and MIX (16%) steers tended to have less SELENOP mRNA than ISe steers, whereas the concentration of hepatic Se did not differ among OSe, ISe, and MIX steers (data not shown). PCA showed that mRNA of SELENOP and hepatic Se concentration were present in the same quadrant but not associated with each other very closely (Figure 6.2). These findings above indicated that SelP synthesis may not be a direct reflection of hepatic Se content.

Methionine sulfoxide reductase B1 (Selenoprotein R)

Selenoprotein R (SelR), also known as methionine sulfoxide reductase B1 (MSRB1) or selenoprotein X, is a zinc-containing stereospecific methionine R sulfoxide reductase. It is predominantly localized in the nucleus and cytosol (Kim and Gladyshev, 2004). SelR is sensitive to dietary Se intake (Novoselov et al., 2005). It is the major mammalian methionine sulfoxide reductase B with the highest activity in liver and kidney (Kim and Gladyshev, 2004). SelR catalyzes the specific reduction of R-isomer of oxidized methionine in proteins. Therefore, it is required for repair of oxidative damaged proteins (Kryukov et al., 2002). In addition, it is reported that SelR is important for

regulation of cellular functions by reduction of methionine residues of regulatory proteins (Lee et al., 2013). That both OSe and MIX steers had more MSRB1 than ISe steers (Table 6.1) suggests that OSe and MIX steers may handle oxidative-damaged proteins better than ISe steers in pituitary.

15-kDa selenoprotein (Selenoprotein F)

The 15-kDa selenoprotein (Sep15) is one of the earliest identified selenoproteins. It is named for its molecular mass (Behne et al., 1997). A single Sec residue is located in the middle of the protein (Gladyshev et al., 1998). Sep15 is expressed in a wide range of tissues in mammals, with the highest levels in liver, kidney, prostate, and testis (Kumaraswamy et al., 2000). Its expression is sensitive to dietary Se intake (Ferguson et al., 2006). Sep15 has a thioredoxin-like domain, and N-terminal signal peptide, which is consistent with its location to the ER (Labunskyy et al., 2014). In addition, Sep15 interacts with the UDP-glucose: glycoprotein glycosyltransferases (UGGT) to form a tight complex. UGGT is an ER-resident chaperone which is involved in N-linked glycoproteins folding in the ER (Hebert et al., 1995; Molinari and Helenius, 1999). Hence, Sep15 is involved in disulfide-bond formation and quality control of some glycoproteins in the ER (Labunskyy et al., 2007). Moreover, studies also suggest that Sep15 may be involved in cancer etiology in various types of tissues (Kumaraswamy et al., 2000; Hu et al., 2001; Apostolou et al., 2004; Nasr et al., 2004; Irons et al., 2010). That the pituitaries of OSe steers tended to possess more SEP15 mRNA than ISe steers (Table 6.1) suggests that OSe steers may have had a better quality control of protein-folding than ISe steers.

Selenophosphate synthetase 2

Selenophosphate synthetase 2 (SPS2, encoded by SEPHS2) is homologous to selenophosphate synthetase 1 (SPS1) in mammals. However, unlike SPS1 in which the putative active center Sec is replaced by threonine, SPS2 belongs to the selenoprotein family (Low et al., 1995). Selenophosphate synthetase 2 (SPS2) catalyzes the conversion of selenide to active Se donor selenophosphate, which is required for Sec biosynthesis (Xu et al., 2007). SPS2 is an interesting selenoprotein in that it possibly regulates its own biosynthesis (Guimarães et al., 1996), thereby playing an auto-regulatory role in selenoprotein synthesis (Kim et al., 1997). The significance of a reduced content of SEPHS2 mRNA in OSe and MIX steers (Table 6.1) is unclear. Although it is a necessary component for the Sec synthesis machinery, whether SPS2 regulates expression of other selenoproteins and other aspects of SPS2 biological function remains to be determined.

Other selenoproteins

The mRNA content of selenoprotein M, N, O, and S were differentially expressed in pituitaries (Table 6.1), as were selenoproteins O, S, T in livers (Table 6.2), of Se treatment groups. However, their biochemical functions of these proteins have not been definitively determined. Selenoprotein M is proposed to be involved in neuroprotection against oxidative damage by H_2O_2 and regulation of Ca^{2+} release from ER in neurons (Reeves et al., 2010). Selenoprotein N plays a role in maintenance of muscle progenitor satellite cells and regeneration of impaired skeletal muscle (Castets et al., 2011), but its role in other tissues is still in a mystery. Selenoprotein S is localized to the ER membrane and proposed to be involved in degradation of misfolded proteins (Labunskyy et al., 2014) and regulation of inflammatory and immune response (Curran et al., 2005; Gao et

al., 2006). The proposed roles that selenoprotein T is involved in include: endocrine homeostasis, brain development and function, and neuroprotection (Youssef et al., 2018).

Both microarray and RT-PCR analyses revealed no difference in mRNA expression of selenoprotein I, K, V, and W, in both pituitary and liver tissues among Se treatment groups. In addition, microarray analysis found selenoprotein H mRNA unchanged in both pituitary and liver (Table 6.1 and 6.2). SelW may play a role in redox-related process regulation because it binds GSH to form a complex with very high affinity (Beilstein et al., 1996). Although the expression of SelW is reported to be regulated by the availability of dietary Se (Howard et al., 2013) and by form of Se (Matthews et al., 2015), in this study the form of Se did not affect expression of SELENOW in either pituitary of liver tissue.

In summary, consumption of 3 mg Se/d in VM mixes as OSe, MIX, or ISe differentially affected the expression of selenoprotein profiles in both pituitaries and livers of growing beef steers commonly grazing an endophyte-infected tall fescue pasture. Most of the affected selenoproteins were either up-regulated by OSe or MIX supplementation relative to ISe supplementation. The change in selenoprotein gene expression in pituitaries indicates that OSe steers have a greater potential capacity to manage against oxidative damage, maintain cellular redox balance, and have a better quality control of protein-folding in their pituitaries than ISe steers. The change in selenoprotein gene expression by the liver indicates that MIX steers have a greater redox signaling capacity and capacity to manage oxidative damage than ISe steers.

Table 6.1. Microarray and real-time RT-PCR (RT-PCR) analyses of the effect of consuming 3 mg Se/d in vitamin-mineral mixes as either sodium selenite (ISe), SEL-PLEX (OSe), or a 1:1 mix of ISe and OSe (MIX) during summer-long grazing of endophyte-infected tall fescue on pituitary selenoprotein gene expression by growing beef steers.

Gene	Gene Name	Microarray[1]				RT-PCR[2]			
		Treatment[3,4]			*P*-value	Treatment[3,4]			*P*-value
		ISe	MIX	OSe		ISe	MIX	OSe	
DIO1	Iodothyronine Deiodinase 1	1.00	1.04	1.07	0.523	1.17	1.37	0.93	0.404
DIO2	Iodothyronine Deiodinase 2	1.00	1.15	1.13	0.456	1.09	1.42	1.42	0.393
DIO3	Iodothyronine Deiodinase 3	1.00	0.84	0.95	0.118	1.08	1.17	1.47	0.219
GPX1	Glutathione Peroxidase 1	1.00	1.11	1.06	0.474	1.06	1.09	1.04	0.955
GPX2	Glutathione Peroxidase 2	1.00	0.96	0.89	0.483	1.03	0.91	0.85	0.351
GPX3	Glutathione Peroxidase 3	1.00[a]	1.46[b]	1.57[b]	0.020	1.17[a]	1.86[a]	3.28[b]	0.010
GPX4	Glutathione Peroxidase 4	1.00[a]	1.21[b]	1.23[b]	0.004	1.03[a]	1.32[a][b]	1.56[b]	0.052
GPX6	Glutathione Peroxidase 6	1.00	0.95	0.95	0.780	1.14[a]	0.53[b]	0.79[a][b]	0.028
LRP2 (Megalin)	LDL Receptor Related Protein 2	1.00[a]	0.92[b]	0.99[a]	0.038	1.08[a]	0.53[b]	0.74[a][b]	0.016
LRP8 (APOER2)	LDL Receptor Related Protein 8	1.00[a]	1.26[b]	1.14[a][b]	0.020	1.01[a]	1.67[b]	1.34[a][b]	0.028
MSRB1 (SEPX1)	Methionine Sulfoxide Reductase B1	1.00[a]	1.23[b]	1.19[b]	0.011	1.05[a]	1.81[b]	1.92[b]	0.053
SELENOF (SEP15)	Selenoprotein F	1.00	0.99	0.96	0.409	1.02[a]	1.24[a][b]	1.37[b]	0.082
SELENOH	Selenoprotein H	1.00	1.16	1.06	0.192	NA	NA	NA	NA
SELENOI (EPT1)	Selenoprotein I	1.00	1.10	0.96	0.135	1.06	1.41	1.07	0.192

Table 6.1 (continued)

SELENOK	Selenoprotein K	1.00	1.10	1.12	0.165	1.19	1.57	1.70	0.550
SELENOM	Selenoprotein M	1.00[a]	1.13[b]	1.26[b]	0.077	3.19[a]	7.57[b]	11.69[b]	0.005
SELENON	Selenoprotein N	1.00[a]	1.13[b]	1.06[a]	0.013	1.10[a]	2.20[b]	1.77[a]	0.015
SELENOO	Selenoprotein O	NA	NA	NA	NA	1.01[a]	1.29[a]	1.64[b]	0.033
SELENOP	Selenoprotein P	1.00	1.00	1.00	0.982	1.01	1.11	1.10	0.565
SELENOS	Selenoprotein S	1.00	1.08	1.02	0.277	1.02[a]	1.25[a]	1.41[b]	0.067
SELENOT	Selenoprotein T	1.00	1.03	1.00	0.489	1.01	1.08	1.17	0.461
SELENOV	Selenoprotein V	1.00	1.01	1.05	0.850	1.04	1.12	1.12	0.810
SELENOW	Selenoprotein W	1.00	1.03	1.01	0.910	1.02	1.10	1.03	0.830
SEPHS1	Selenophosphate Synthetase 1	1.00	1.03	0.99	0.548	1.07	1.03	1.03	0.965
SEPHS2	Selenophosphate Synthetase 2	1.00[a]	0.93[a]	0.88[b]	0.045	1.01[a]	0.74[b]	0.81[b]	0.019
TXNRD1	Thioredoxin Reductase 1	1.00[a]	1.12[b]	0.97[a]	0.001	1.00[a]	1.42[b]	0.98[a]	0.005
TXNRD2	Thioredoxin Reductase 2	1.00[a]	1.15[b]	1.03[a]	0.036	1.05[a]	1.82[b]	1.66[a]	0.084
TXNRD3	Thioredoxin Reductase 3	1.00	0.92	0.95	0.286	1.02	1.35	1.49	0.130

[1]The abundance of gene transcripts are reported relative to the mean expression of the ISe treatment group (untransformed microarray data).

[2]The abundance of gene transcripts are reported relative to the geometric mean expression of the reference genes.

[3]Values are least squares means (n = 6 for ISe, n = 7 for OSe and MIX)

[4]Means within a row that lack a common letter differ ($P < 0.1$).

Table 6.2. Microarray and real-time RT-PCR (RT-PCR) analyses of the effect on liver selenoprotein gene expression by growing beef steers consuming 3 mg Se/d in vitamin-mineral mixes as either sodium selenite (ISe), SEL-PLEX (OSe), or a 1:1 mix of ISe and OSe (MIX) during summer-long grazing of endophyte-infected tall fescue.

Gene	Gene Name	Microarray[1]				RT-PCR[2]			
		Treatment[3,4]			*P*-value	Treatment[3,4]			*P*-value
		ISe	MIX	OSe		ISe	MIX	OSe	
DIO1	Iodothyronine Deiodinase 1	1.00	1.32	1.57	0.239	1.22	1.89	1.87	0.382
DIO2	Iodothyronine Deiodinase 2	1.00[a]	1.11[b]	1.03[ab]	0.075	1.22	1.13	1.12	0.900
DIO3	Iodothyronine Deiodinase 3	1.00	1.04	0.93	0.355	1.04[a]	0.67[b]	0.81[ab]	0.092
GPX1	Glutathione Peroxidase 1	1.00	0.93	0.91	0.280	1.02	0.96	0.91	0.730
GPX2	Glutathione Peroxidase 2	1.00	0.99	1.03	0.724	1.04	0.77	1.09	0.133
GPX3	Glutathione Peroxidase 3	1.00	0.68	1.16	0.221	1.35	0.55	0.87	0.210
GPX4	Glutathione Peroxidase 4	1.00	1.22	1.08	0.096	1.01[a]	1.57[b]	1.32[ab]	0.067
GPX6	Glutathione Peroxidase 6	1.00[a]	1.21[b]	1.17[b]	0.020	1.79	0.66	0.98	0.275
LRP2 (Megalin)	LDL Receptor Related Protein 2	1.00	0.90	0.85	0.312	1.81	1.61	0.74	0.195
LRP8 (APOER2)	LDL Receptor Related Protein 8	1.00	1.02	1.03	0.877	1.04	0.81	1.15	0.282
MSRB1 (SEPX1)	Methionine Sulfoxide Reductase B1	1.00	1.05	0.98	0.505	1.01	1.00	0.87	0.472
SELENOF (SEP15)	Selenoprotein F	1.00	1.07	1.06	0.257	1.01	0.94	0.85	0.187
SELENOH	Selenoprotein H	1.00	0.92	0.94	0.394	NA	NA	NA	NA
SELENOI (EPT1)	Selenoprotein I	1.00	1.00	1.00	1.000	1.02	1.07	1.01	0.873
SELENOK	Selenoprotein K	1.00	1.03	1.03	0.919	1.14	1.17	1.19	0.990

Table 6.2 (continued)

SELENOM	Selenoprotein M	1.00[a]	0.89[b]	0.98[a][b]	0.080	3.44	3.03	3.28	0.939
SELENON	Selenoprotein N	1.00	0.96	1.03	0.150	1.00	1.12	1.16	0.388
SELENOO	Selenoprotein O	NA	NA	NA	NA	1.01[a]	1.21[b]	1.01[a]	0.040
SELENOP	Selenoprotein P	1.00	0.93	0.96	0.127	1.01[a]	0.85[b]	0.82[b]	0.058
SELENOS	Selenoprotein S	1.00[a]	1.19[b]	1.05[a][b]	0.079	1.02[a]	1.47[b]	1.13[a]	0.001
SELENOT	Selenoprotein T	1.00[a]	1.09[b]	1.09[b]	0.021	1.00[a]	1.35[b]	1.40[b]	0.005
SELENOV	Selenoprotein V	1.00	0.92	1.01	0.511	1.17	1.20	1.01	0.769
SELENOW	Selenoprotein W	1.00	0.91	0.91	0.186	1.01	0.86	0.85	0.445
SEPHS1	Selenophosphate Synthetase 1	1.00	1.06	1.01	0.658	1.01	1.24	1.10	0.161
SEPHS2	Selenophosphate Synthetase 2	1.00	1.02	1.03	0.890	1.02[a][b]	1.18[a]	0.96[b]	0.092
TXNRD1	Thioredoxin Reductase 1	1.00[a]	1.15[b]	1.13[a][b]	0.081	1.01[a]	1.36[b]	1.37[b]	0.049
TXNRD2	Thioredoxin Reductase 2	1.00	0.98	0.97	0.902	1.01[a]	0.95[a]	0.80[b]	0.021
TXNRD3	Thioredoxin Reductase 3	1.00	1.05	1.05	0.446	1.02	1.04	0.98	0.886

[1]The abundance of gene transcripts are reported relative to the mean expression of the ISe treatment group (untransformed microarray data).

[2]The abundance of gene transcripts are reported relative to the geometric mean expression of the reference genes.

[3]Values are least squares means (n = 6 for ISe, n = 8 for OSe and MIX)

[4]Means within a row that lack a common letter differ ($P < 0.1$).

Table 6.3. Primer sets used for quantitative real-time RT-PCR analysis of the selected differentially expressed genes and reference genes.

Gene	Gene Name	Template Accession number[1]	Oligonucleotide Primer Design (5' to 3' direction)	Amplicon length (bp)	Product identity (%)[2]
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_001034034.2	F: ACATCAAGTGGGGTGATGCT R: GGCATTGCTGACAATCTTGA	201	99%
PCK2	Phosphoenolpyruvate carboxykinase 2	NM_001205594.1	F: GCAAGCTGTGGATGAGAGGT R: TGACAAAGTCGCCATCTCCC	203	99%
RPS11	Ribosomal protein S11	NM_001024568.2	F: AAGATGGCGGACATTCAGAC R: GCCCTCGAATGGAGACATTA	214	99%
SDHA	Succinate dehydrogenase complex flavoprotein subunit A	NM_174178.2	F: GCAGAACCTGATGCTTTGTG R: CGTAGGAGAGCGTGTGCTT	185	99%
TFRC	Transferrin receptor	NM_001206577.1	F: CCAGGTTTAGTCTGGCTCGG R: GGTCTGCCCAGAATATGCGA	339	99%

Table 6.3 (continued)

UBC	Ubiquitin C	NM_001206307.1	F: TAGGGGTGGGTTAGAGTTCAAG R: ACCACCTCCCTGCTGGTATT	258	100%
DIO1	Iodothyronine deiodinase 1	NM_001122593.2	F: ATTGCCCAGTTCTAGGTGCC R: CAGAAGGAAATGCAGCGTGT	273	100%
DIO2	Iodothyronine deiodinase 2	NM_001010992.4	F: GATGGGCATCCTCAGCGTAG R: TTCTCCTGGGCACCATTTCC	315	100%
DIO3	Iodothyronine deiodinase 3	NM_001010993.3	F: AAGTGGAGCTCAACAGCGAT R: AGTCGAGGATGTGCTGGTTC	213	100%
GPX1	Glutathione peroxidase 1	NM_174076.3	F: AAACGCCAAGAACGAGGAGA R: GTCGGTCATGAGAGCAGTGG	184	99%
GPX2	Glutathione peroxidase 2	NM_001163139.2	F: AACAGCCTCAAGTACGTCCG R: TCGGTCATGAGGGAAAACGG	158	100%

Table 6.3 (continued)

GPX3	Glutathione peroxidase 3	NM_174077.4	F: AGGAGAAGTCGAAGACGGACT R: CTCAGTAGCTGGCCACGTTGA	147	100%
GPX4	Glutathione peroxidase 4	NM_174770.3	F: GATCAAAGAGTTCGCCGCTG R: CCATACCGCTTCACCACACA	198	100%
GPX6	Glutathione peroxidase 6	NM_001163142.1	F: CACTGTTCCTGGTCGGCTTA R: CCCAGCACAACTACACCGAA	259	99%
LRP2	LDL receptor related protein 2	XM_015462263.1	F: GTGGTTTGGGTTACCGTTGC R: GGCACCCTGTTAGCTGTGAT	304	98%
LRP8	LDL receptor related protein 8	NM_001097565.1	F: AGCCACCCTTTTGGGATAGC R: AAGGCACAGGTACTCACAGC	231	98%
MSRB1	Methionine sulfoxide reductase B1	NM_001034810.2	F: GAACCACTTTGAGCCGGGTA R: GGCCATCGTTCAGGAACTCA	221	100%

Table 6.3 (continued)

SELENOF	Selenoprotein F	NM_001034759.2	F: GGGAGATGCCTGATGTGAGT R: TCCAGTGCCTGATCCAAAGC	174	100%
SELENOH	Selenoprotein H	NM_001321327.1	F: CACGAGCTGACGAGTCTACG R: CTTCTTCAGCTCCTCCAGCA	235	100%
SELENOI	Selenoprotein I	NM_001075257.2	F: TACCTGACGTGTTGGCAGAC R: CACTTGTGAAAAAGGCCGCA	341	100%
SELENOK	Selenoprotein K	NM_001037489.3	F: CCGTTTTGTCGATTCACGGC R: CAGATGAGCTTCCGTAGCCT	278	100%
SELENOM	Selenoprotein M	NM_001163171.2	F: CCCACTCTACCACAACCTGG R: ACCTAAAGGTCTGCGTGGTC	249	100%
SELENON	Selenoprotein N	NM_001114976.2	F: GTGGCCATGTACCCCTTCAA R: GGGATGGGTTCTCCTGGTTG	265	100%

Table 6.3 (continued)

SELENOO	Selenoprotein O	NM_001163193.2	F: TGGACAGGTATGACCCCGAT R: ATCTTCTGCAGGTAGTGCCG	202	100%
SELENOP	Selenoprotein P	NM_174459.3	F: TCAGGTCTTCATCACCACCA R: GTGGCAACAGCAGCTACTCA	201	100%
SELENOS	Selenoprotein S	NM_001046114.3	F: CCCACCCTCGAGACCGA R: GCCCAGGACTGTCTTCTTCC	394	100%
SELENOT	Selenoprotein T	NM_001103103.2	F: TGGTCACCTTCCATCCATGC R: AAGAGGTACAACGAGCCTGC	240	100%
SELENOV	Selenoprotein V	NM_001163244.2	F: ACTCCATTGGCCACCGATTT R: AGGCCACAGTAAACCACTCG	224	100%
SELENOW	Selenoprotein W	NM_001163225.1	F: AGTGTTCGTAGCGGGAAAGC R: CGCGAGAACATCAGGGAAGG	233	98%

Table 6.3 (continued)

SEPHS1	Selenophosphate synthetase 1	NM_001075316.1	F: CAAAGCGAACCGGTGGATCT R: GAGGTCACTGAGGACGTTGG	422	99%
SEPHS2	Selenophosphate synthetase 2	NM_001114732.2	F: GATCCCTACATGATGGGGCG R: GTTTACCACCGTTTGCCCAC	219	100%
TXNRD1	Thioredoxin reductase 1	NM_174625.3	F: AAGGCCGCGTTATTTGGGTA R: CCTGGTGTCCCTGCTTCAAT	306	98%
TXNRD2	Thioredoxin reductase 2	NM_174626.2	F: CAAATGGCTTCGCTGGTCAC R: TTCGTATGCACACCAGCCTT	230	100%
TXNRD3	Thioredoxin reductase 3	XM_015468824.1	F: CGGCGTATGACTACGACCTC R: GACTGTACTCCCAGCCGAAC	249	99%

[1]The contents in the parentheses associated with each gene symbol are the accession numbers of the sequences retrieved from the NCBI RefSeq database and used as templates for designing primers and probes.

[2]All the real-time RT-PCR products were validated by sequencing. The identity values (%) presented are the base-pair ratios between the number of identical base pairs and the total amplicon length.

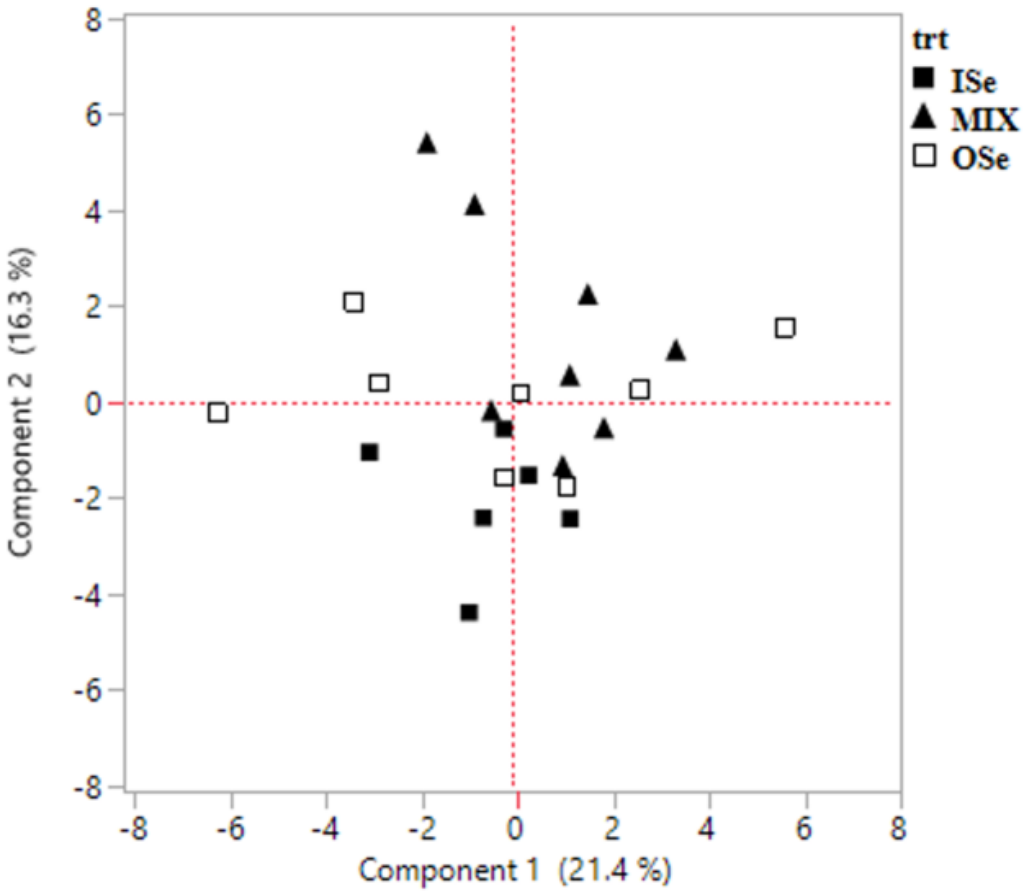


Figure 6.1. Score plot from principal component analysis of steer (ISe, OSe, and MIX) parameters showing the correlation of the first two principal components (Components 1 and 2) among Se concentration, glutathione (GSH) content, glutamine synthetase (GS) activity, and relative mRNA abundance of selenoproteins in liver.

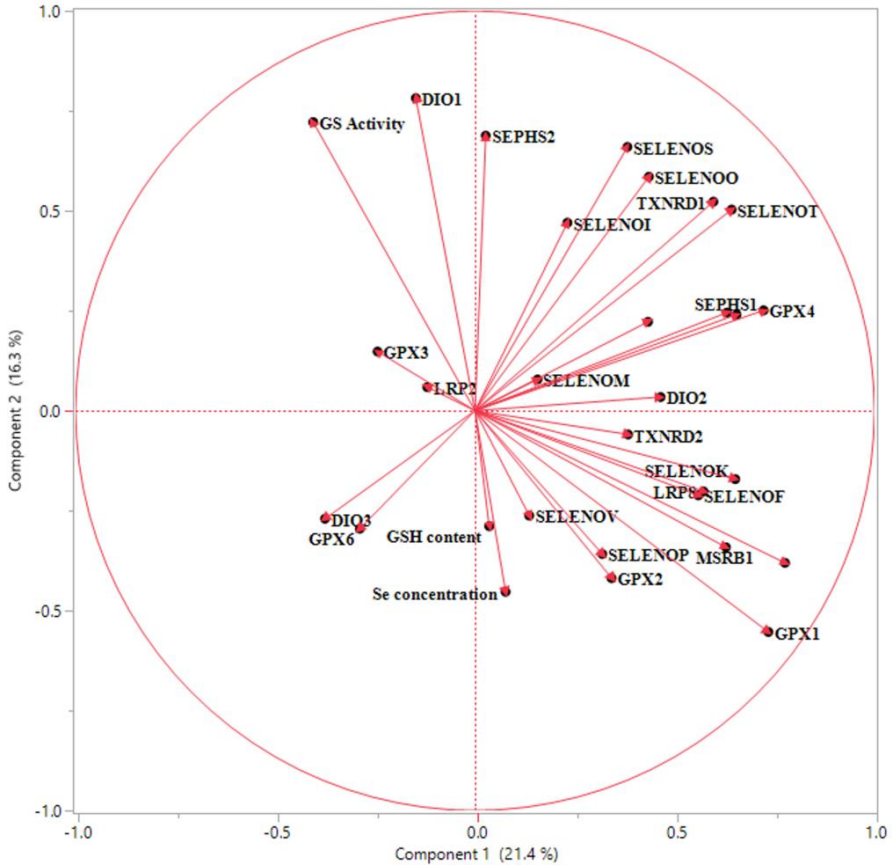


Figure 6.2. Loading plot from principal component analysis of steer (ISe, OSe, and MIX) parameters showing the correlation of the first two principal components (Components 1 and 2) among Se concentration, glutathione (GSH) content, glutamine synthetase (GS) activity, and relative mRNA abundance of selenoproteins in liver.

***GAPDH*:**

ACATCAATGGGGTGATGCTGGTGCTGAGTATGTGGTGGAGTCCACTGGGGTC
TTCACTACCATGGAGAAGGCTGGGGCTCACTTGAAGGGTGGCGCCAAGAGGG
TCATCATCTCTGCACCTTCTGCCGATGCCCCCATGTTGTGATGGGCGTGAACC
ACGAGAAGTATAACAACACCCTCAAGATTGTCAGCAATGCC

***PCK2*:**

GCAAGCTGTGGATGAGAGGTTTCCAGGCTGCATGCTGKGCCSAACATGTACG
TGATTCCGTTCAGCATGGGTCCCGTGGGCTCCCCGCTGTCCCGCATCGGAGTG
CAGCTCACGGACTCTGCCTACGTGGTGGCAAGCATGCGGATTATGACTCGGT
TGGGGACACCTGTGCTTCAGGCCCTGGGAGATGGCGACTTTGTCA

***RPS11*:**

AAGATGGCGGACATTCAGACAGAACGTGCGTACCAAAAGCAACCGACCATC
TTTCAAAATAAAAAGAGGGTCCTGCTTGGAGAAACTGGCAAAGAAAAGCTCC
CTCGATACTACAAGAACATTGGTCTGGGCTTCAAGACTCCAAAGGAGGCCAT
CGAGGGCACCTACATTGACAAGAAATGCCCTTTTACGGGTAATGTCTCATTCG
AGGGC

***SDHA*:**

CGTAGGAGAGCGTGTGCTTCCTCCAGTGCTGCTCAAAGGGCTTCTTCTGCTGC
CCCTGGATGGGCTTGGAGTAATCGTACTCGTCAACCCTCTCCTTGAAGTCCTC
CCTGGCGTGGGCGCCGCGCGACTCCTtCCGGGCCTCCGCTCCGTAGATGGTCT
GCAGAGCACAAAGCATCAGGTTCTGC

***TFRC*:**

CCAGGTTTAGTCTGGCTCGGCAAGTAGATGGTGATAACAGTCATGTGGAGAT
GAAATTAGCTGCAGATGAAGAAGAANAATGTTGACAGTAACATGAGGGGCA
ACCAAACCAGTATCGCAAAACCGAAAAGGTTAAATGGATATGTCTGCTACGG
GATCATTGCTGTAATCGTCTTTTTCTTGATTGGATTTATGATTGGCTACTTGGG
CTATTGTAGACGTGTGGAATCACAAGATTGTGGGAAAGAGGCAGGAACACA
GCCTTCGTGCCCAGGAGGAGACAGAAACTTTCGAATCAGAAGAGCAACTCCC
TGGAGTACCTCGCATATTCTGGGCAGACC

***UBC*:**

TAGGGGTGGGTTAGAGTTCAAGGTTTTTGTTCTACCAGATGTTTTAGTAGTAA
TCTGGAGGTAAGAAATGTCAAGAAAACATGGCCTTAATTAGAACTGTAGTGG
GTGAGTATAAATAAAAAATTTGGAGGTTGTAGTTAGAATTCTCCATATGTAC
ACTCATATGTAGATCTACTTATAAGCTACTGATTTTTAAAAGCACACGTTTGG
GAGTTGTGCTTAAGAGTGGGAAAGTTTCTGGAATACCAGCAGGGAGGT

DIO1:

ATTGCCCAGTTCTAGGTGCCCAACCTGAGGGCCCCTTAAGGCTTGGTTTGACC
CCCATCCCAGCTGACATTTACCTCTTGACCTGTGTTCCCAGTTGAATCACTAG
CCTGGATTTTTCTGATCCAAGCAAACAATTGTTGCAATGAGAAAATGAAGCC
ACAAGTAAGCTGAGTATTAAAGAAGGTATAATTTCGTACTGCTCCTACCATG
GAGACTGTGTTCATTGCACAGCATTCTAAGAATGAGAGCAGGTACACGCTGC
ATTTCCTTCTG

DIO2:

GATGGGCATCCTCAGCGTAGACTTGCTGATCACACTGCAGATTCTGCCAGTTT
TTTTCTCCAACTGCCTCTTCCTGGCGCTCTATGACTCGGTCATTCTCCTCAAGC
ACGTGGTGCTGCTGCTGAGCCGCTCCAAGTCCACTCGCGGGCAGTGGAGGCG
CATGCTGACCTCAGAAGGAATGCGCTGCATCTGGAAAAGCTTCCTCCTCGAC
GCCTACAAACAGGTGAAACTGGGTGAAGATGCCCCCAATTCCAGCGTGGTGC
ATGTCTCCAGTCCGGAAGGAGGTGACACCAGTGGAAATGGTGCCCAGGAGA
A

DIO3:

AAGTGGAGCTCAACAGCGATGGCGAGGAGGTGCCCCCCGACGACCCGCCTGT
CTGCGTGTCCGACGACAACCGCCTGTGCACCTTGGCGTCGCTGAGGGCCGTC
TGGCACGGCCAGAAGTTGGATTTCTTCAAGCAGGCGCATGAAGGCGGGCCGG
CGCCCAACTCTGAGGTGGTCCTGCCCGACGGCTTCCAGAACCAGCACATCCT
CGACT

GPX1:

AAACGCCAAGAACGAGGAGATCCTGAATTGCCTGAAGTACGTCCGACCAGG
CGGCGGGTTCGAGCCCAACTTTATGCTCTTCGAAAAGTGCGAGGTGAATGGC
GAGAAGGCGCATCCGCTCTTCGCCTCCTTCGGGAGGTTCTGCCCACGCCAAG
TGACGACGCCACTGCTCTCATGACCGAC

GPX2:

AACAGCCTCAAGTACGTCCGCCCCGGGGGTGGGTTCCAGCCCACCTTCACCC
TTGTCCAGAAGTGTGATGTGAATGGTCAGAATGAGCATCCTGTCTTTGCCTAC
CTGAAGGATAAGCTCCCCTACCCTTATGATGACCCGTTTTCCCTCATGACCGA

GPX3:

AGGAGAAGTCGAAGACGGACTGCCACGCTGGTGTGGGTGGCACCATCTATGA
GTACGGGGCCCTCACCATCGATGGGGAGGAGTACATCCCCTTTAAGCAGTAC
GCTGGCAAATACATCCTCTTCGTCAACGTGGCCAGCTACTGAG

GPX4:

GATCAAAGAGTTCGCCGCTGGCTATAACGTCAAATTCGATTTGTTCAGCAAG
ATCTGTGTAAATGGGGACGACGCCCACCCTCTGTGGAAATGGATGAAAGTCC

AGCCCAAGGGGAGAGGCATGCTGGGAAACGCCATCAAATGGAACTTCACCA
AGTTCCTCATTGACAAGAACGGCTGTGTGGTGAAGCGGTATGG

GPX6:

CACTGTTCCTGGTCGCTTAGCTCAGCTGACTCCGAAGCAGCAACAGATGAAG
GTGGATTGCTATAAGGGGGTGACAGGCACCATCTATGAGTATGGAGCCCTCA
CCCTCAATGGTGAGGAGTATATCCAGTTCAAGCAGTATGTGGGCAAGCATGT
CCTGTTTGTCAATGTGGCCACCTATTGAGGCTTGACAGCTCAGTTCCAGAACT
GAATGCACTACAGGAGGAGCTGAAGCCTTTCGGTGTAGTTGTGCTGGG

LRP2:

GTGGTTTGGGTTACCGTTGCAAGTGTAGGCTTTGCGAACTTCGGATTGGGATG
ACTATCACTGTGTTGCTGCTGAGCGGTTTCTGCTCTTTTCATCTACATCTGGCT
GTTCGTGGCATCCCACTCACCCTCTCTCACCAGACGGAGGTCATCCTTCCAGT
GACAGGATCTTCTTCCATCTTCCTTGGGATCGATTTTGATGCCCGCGAGAAGG
CTATCTTTTTTTCAGATACAAAGAAAAACATTATTTATAGACAAAAGCTCGAT
GGTACAGGAAGAGAAATTATCACAGCTAACAGGGTGCC

LRP8:

AGCCACCCTTTTGGGATAGCTGTGTTTGAGGACAAVGTGTTCTGGACGGACCT
GGAGAATGAGGCCATTTTCAGTGCAAATCGGCTCAATGGCCTGGAAATCTCC
GTCCTAGCTGAGAACCTCAACAACCCGCATGATATAGTCATCTTCCATGAGCT
GAAGCAGCCAAGAGCTGCAGATGCCTGAGCGAGGCCCAGCCCAATGGAGGC
TGTGAGTACCTGTGCCTT

MSRB1:

GAACCACTTTGAGCCGGGTATCTACGTGTGTGCCAAGTGTGGCTATGAGCTCT
TCTCCAGCCGCTCCAAGTACGCACACTCATCCCCATGGCCGGCGTTCACTGAG
ACCATCCATGCTGACAGTGTGGCCAAGCGGCCAGAGCACAATCGGCCTGGAG
CCATAAAGGTATCCTGTGGCAGGTGTGGCAACGGGCTGGGCCATGAGTTCCT
GAACGATGGCC

SELENOF:

GGGAGATGCCTGATGTGAGTTATCGAAAGGACCCAGTGACTTCATATTTACA
TCAAGTGTAAAGTTTTTGCTCACCTTTAAGCTTACATATGCCCGTAGTTAATG
CCCGTCTCCTCAGAATAAGAGACTTGATGGCAGCGGCCTTTAGCAGCCTGCTT
TGGATCAGGCACTGGA

SELENOH:

CACGAGCTGACGAGTCTACGGGCGCAACGCCGCGGCCCTGAGCCAGGCGCtG
CGCCTGCAGGCCCCCGAGCTGACGGTGAAGGTGAACCCCGCCAGGCCGCGG
AGGGGCAGCTTCGAGGTGACGTTGCTGCGCGCCGACGGCAGCAGCGCGGAG

CTCTGGACGGGTCTTAAGAAGGGGCCCCCACGCAAACTCAAGTTTCCGGAGC
CTCACGTGGTGCTGGAGGAGCTGAAGAAG

SELENOI:

TACCTGACGTGTTGGCAGACAGTTGCTTTAGGATATATGGTAAATCATGGTTG
TCTAGCTTTTCCAGAGAAAGTGGCAGTGTGTTTACTTTTTCCTTTGTGTGGAG
CCGTGGGAATTGGACTTATTGGCTAATTCATCTCCATTGTTTCTGTGGTGCAC
GGTAAACAACCTTTTAAAGCTGCATACTAGTAATGCTTTCCTTTTTAATAAAT
ATGCGAAAGACAGTAATCTAGCCAATTGTTTTTAAGTAGATGCTTGCTCTTTT
TTCAGACATACCTTAAATGAACTAATATTTCGGGTAGCATAAGAAGTTTTGGC
ATTTGCGGCCTTTTTCACAAGTG

SELENOK:

CCGTTTTGTCGATTCACGGCCTGTGATGTAAGAGGGAGGCGCGGAGGGAGAT
AAGGAATCCAAGAGGGGTCAGGCGTCGCCGTCGCCCTGAAGACGGAGGAGG
GGCAAGATGGTTTACATCTCGAATGGACAAGTGTTGGACAGCAGGAGTCAGT
CCCCATGGAGATTATCTTTTATAACAGATTTCTTCTGGGGAATAGCTGAGTTT
GTGGTTTTGTTTTTCAGAACTCTGCTTCAACAAGATGTGAAAAAGAGAAGAG
GCTACGGAAGCTCATCTG

SELENOM:

CCCACTCTACCACAACCTGGTGATGAAACACCTGCCGGGGGCCGACCCAGAG
CTCGTGTTGCTCGGCCACCGCTTTGAGGAACTGGAGCGAATTCCACTCAGCG
ACATGACCCGCGAGGAGATCAACGCGCTGGTGCAGGAGCTCGGCTTCTACCG
CAAGGCGTCGCCCGACGAGCCTGTGCCCCCGGAGTACCTTCGGGCGCCCGCT
AGGCCCGCCGGAGACGCTCCTGACCACGCAGACCTTTAGGT

SELENON:

GTGGCCATGTACCCCTTCAAGAAGGTCACCTACCTGCCGTTCACCGAGGCCTT
TGACCAAGCCAAGGCCAAGAACAAGCTGGTGCACTCAATCCTGCTGTGGGGG
GCCCTGGACGACCAGTCCTGCTGAGGTTCGGGGCGGACTCTCCGGGAGACCG
TCCTGGAAAGTTCGCCCATCCTCGCCCTCCTCAATGAGAGCTTCATCAGCACC
TGGTCCCTGGTGAAGGAGCTGGAGGACCTGCAGAACAACCAGGAGAACCCA
TCCC

SELENOO:

TGGACAGGTATGACCCCGATCACGTGTGCAACGCCTCCGACACCGCCGGGCG
CTACTCGTACAGCAAGCAGCCCGAGGTGTGCAAGTGGAACCTGCAGAAGCTG
GCCGAGGCCCTGGACCCCGCGCTGCCCCTCGAGCTGGCCGAGGCCATCCTGG
CAGAGGAGTTCGACGCCGAGTTCGGCCGGCACTACCTGCAGAAGAT

SELENOP:

TCAGGTCTTCATCACCACCACCACAGGCACAAGGGTCCCCAAAGACAGGGTC
ACTCAGATAACTGTGATACACCAGTAGGAAGTGAAAGTTTACAACCTTCTCTT
CCACAAAAGAAGCTCTGACGAAAGAGATGCATAAATCAGTTACTCTGACAGT
TTCCCAAAGATTCAGAATCTGCTTTGAGTAGCTGCTGTTGCCAC

SELENOS:

CCCACCCTCGAGACCGAGGGGCTGCGTTTCCTTCACGTCACGGTGGGCTCCCT
GCTGGCCACTTACGGCTGGTACATCGTCTTCAGCTGCATCCTTCTCTACGTGG
TGTTTCAGAAGCTTTCCACCCGGTTGAGGGCCTTGAGGCAGAGGCACTTAGA
TCAAGCTGCCGCTGCTCTGGAACCTGATATTGTTGTTAAACGACAGGAGGCTT
TAGCAGCTGCTCGTTTGAAAATGCAAGAAGAATTAAATGCACAAGTTGAAAA
GCATAAAGAGAAATTAAGACAGCTTGAAGAAGAAAAAAGGAGACAGAAGAT
CGAAATGTGGGATAGCATGCAAGAAGGAAAAAGTTATAAAGGAAATACAAG
AAAGCCTCAGGAAGAAGACAGTCCTGGGC

SELENOT:

TGGTCACCTTCCATCCATGCAACAGCTGGTTCAAATTCTTGACAATGAAATGA
AGCTCAACGTGCATATGGATTCAATCCCACACCATCGATCATAGCCCCAACT
ATCAGCACTGAAAACTCTTTTACATTAAGGGATTTTTGCAAGAGCAGCGTGA
CTGACATTATGAAGGCCTGTACTGAAGACAGCAAGCTGTTAGTACAGACCAG
ATGCTTTCTTGGCAGGCTCGTTGTACCTCTT

SELENOV:

ACTCCATTGGCCACCGATTTACGGTCCCCATCCCTCGGGTCCCCTCTGAGGAC
AGACACATCGACCACCAATTTGATAGCTTCTTCTTCTGGACATGTCCCAGGGA
CGCCCATCCTAGGGGCCATCCAGGCCATCTTACCGGTTCCTGCCACCGCATTA
GCTTCCATCAGTGGGAACCTCAAAGAGGAAAACAAGATCATGATTCGAGTGG
TTTACTGTGGCCT

SELENOW:

AGTGTTTGTAGCGGGAAAGCTGGTTCACTCCAAGAAGGGAGGCGATGGCTAC
GTGGACACGGAGAGCAAGTTTCTGAAGCTGGTGGCCGCCATCAAAGCCGCTT
TGGCTCAGGCCTGATGTGGCCTGAAGGCAGAGACCAGTAATCGTGGCCCAGC
CCCTCTCGGCAGACGCTTCATGACAGGAAGGACGAAAGTCTCTTGGACGCCT
GGTCCTTCCCTGATGTTCTCGCG

SEPHS1:

CAAAGCGAACCGGTGGATCTATAAAGAAACCAGCTGCGTAGGAGAAGGAGG
GGAGGAGCCCGGCCGTGAGGTGTGTGGGCCCAAGAACCATGTCTGCACGGG
AGTCCTTTAACCCGGAAAGTTATGAATTGGACAAGAGTTTCCGGTTAACCAG
ATTCACTGAACTGAAGGGCACTGGCTGCAAAGTGCCCCAAGATGTCCTGCAG
AAATTGCTGGAGTCCTTACAGGAGAACCACTTCCAAGAAGATGAGCAGTTTC
TTGGAGCAGTTATGCCAAGACTTGGCATCGGGATGGACACCTGTGTCATTCCT

CTGAGGCACGGCGGCCTTTCCTTGGTTCAAACCACAGATTACATTTATCCCAT
CGTCGATGACCCNTACATGATGGGCAGGATAGCCTGTGCCAACGTCCTCAGT
GACCTC

SEPHS2:

GATCCCTACATGATGGGGCGCATCGCATGTGCCAACGTGCTGAGTGACCTTT
ACGCGATGGGCATTACTGAGTGTGACAACATGTTGATGCTACTCAGCGTCAG
CCAGAGTATGCCTGAGGAGGAGCGAGAAAAGATAACACCACTCATGATCAA
AGGCTTTCGAGATGCTGCCGAGGAAGGAGGGACTGCAGTGACTGGTGGGCA
AACGGTGGTAAAC

TXNRD1:

AAGGCCGCGTTATTTGGGTATCCCCGGTGACAAAGAATACTGTAtYMGCAGT
GATGATCTTTTCTCTCTACCTTATTGCCCGGGTAAGACCCTGGTGGTTGGAGC
ATCCTATGTTGCTTTGGAATGTGCTGGATTTCTTGCTGGTATTGGTTTAGACGT
CACTGTTATGGTACGATCCATTCTCCTAAGAGGATTTGACCAGGACATGGCCA
ACAAAATTGGTGAACATATGCAAGAACATGGTATCAAGTTCATAAGACAGTG
ACAATAAAAGTTGAACAAATTGAAGCAGGGACACCAGG

TXNRD2:

CAAATGGCTTCGCTGGTCACCGAGCACATGGCTGGCCACGGCACCCGGATCC
TACGGGGCTGCGCCCCCGAGAAGGTAGAGAAACTCCCAGGCCAGCAGCTCC
GCGTCACCTGGGTGGACCTCACCTCGGACCGGAAGGACGCGGGCACCTTTGA
CACGGTGCTCTGGGCCATAGGCCGAGTTCCAGAAACCGCAAGTCTGAATTTG
GAAAAGGCTGGTGTGCATACGAA

TXNRD3:

CGGCGTATGACTACGACCTCATCGTCATCGGTGGGGGCTCCGGGGGCCTCTC
GTGTGCTCAGGAAGCCGCCGTCCTGGGGAGGAAGGTCATGGTGCTGGACTTC
GTGGTCCCGTCCCCCCGTGGCACGGCCTGGGGTCTTGGTGGCACGTGTGTGA
ATGTTGGCTGTATCCCGAAGAAGCTGATGCACCAGGCCGCGCTGCTGGGGCA
GGCACTGACCGACTCCAGGAAGTTCGGCTGGGAGTACAGTC

Figure 6.3. The sequences of the real-time RT-PCR products (5’ to 3’ orientation).

Within a sequence, underlined nucleotides indicate the forward and reverse primer

positions.

CHAPTER 7. Summary and Conclusions

Improvement of growth performance of growing beef steers grazing endophyte-infected tall fescue has been hindered by a lack of fundamental knowledge about how ergot alkaloids exert their deleterious effects on bovine pituitary function. In addition, knowledge regarding the effects of forms of supplemental Se on pituitary function of growing beef steers grazing endophyte-infected tall fescue has not been reported. The overall objective of this dissertation was to determine whether transcriptome profiles differed between whole pituitaries of growing beef steers grazing pastures containing HE or LE amounts of toxic endophyte-infected tall fescue, and to test whether ISe, OSe, vs. MIX in a basal VM mix would differentially alter pituitary transcriptome profiles in growing beef steers grazing endophyte-infected tall fescue.

In the first experiment of the current study (Chapter 4), the findings indicate that anterior pituitary functions were globally impaired in steers consuming high-toxic endophyte-infected tall fescue. In addition to inhibiting the abilities to synthesize and secrete prolactin (a function of lactotrophs), ACTH synthesis capacity (a function of corticotrophs) might have been reduced. Canonical pathway analysis also indicated that growth hormone signaling and GnRH signaling were altered in HE vs. LE steers. With the identification of putative ergot alkaloid sensitive mechanisms within the pituitary gland, this new knowledge may help to develop dietary treatments that ameliorate the effects of ergot alkaloid ingestion. To our knowledge, this is the first report to describe the impart of consumption of toxic tall fescue on bovine pituitary transcriptome.

In the second experiment of the current study (Chapter 5), the findings indicate that consumption of 3 mg Se/d in VM mixes as OSe, MIX, or ISe differentially affected

the expression of genes responsible for the synthesis or release of prolactin and POMC/ACTH/α-MSH, and for mitochondrial function, in the pituitaries of growing beef steers commonly grazing an endophyte-infected tall fescue pasture. Consumption of OSe resulted in greater prolactin synthesis capacity, whereas consumption of MIX resulted in increased prolactin synthesis and release potential, both of which resulted in greater serum prolactin concentrations in OSe and MIX steers vs. ISe steers, respectively. In addition, consumption of OSe resulted in greater POMC/ACTH/α-MSH synthesis potential, and a better capacity to manage against mitochondrial dysfunction and oxidative stress, than did consumption of MIX or ISe forms of Se. To our knowledge, this is the first report to evaluate the impact of forms of supplemental Se on bovine pituitary transcriptome.

In the third experiment of the current study (Chapter 6), the findings indicate that consumption of 3 mg Se/d in VM mixes as OSe, MIX, or ISe differentially affected the expression of selenoprotein profiles in both pituitaries and livers of growing beef steers commonly grazing an endophyte-infected tall fescue pasture. Compared with liver, the pituitary is more responsive to Se treatment in terms of change in expressions of selenoprotein. For most of the affected selenoproteins, they were either up-regulated by OSe or MIX supplementation when compared to ISe supplementation. Generally, OSe steers may manage against oxidative damage, maintain cellular redox balance, and have a better quality control of protein-folding in their pituitaries than ISe steers. Meanwhile, MIX steers may have more thyroid hormone T3 and better redox signaling, and handle oxidative damage better in livers than ISe steers. This is the first time to describe the

effects on forms of supplemental Se on bovine selenoprotein expression profile in pituitary and liver.

In conclusion, these findings above suggest that consumption of endophyte-infected tall fescue negatively impact bovine pituitary function (e.g. prolactin and ACTH synthesis), and inclusion of the organic form of Se in free-choice vitamin-mineral mixes can ameliorate the negative impact of fescue toxicosis on beef cattle by restoration of both prolactin and POMC/ACTH synthesis capacities to a certain extent. In addition, by identification of genes that are susceptible to ergot alkaloids contained in endophyte-infected tall fescue, a larger implication of this research may be that it allows for selective breeding for genotypes with a higher resistance to endophyte toxicosis.

APPENDIX. Example of SAS Analysis

Analysis of mRNA expression of UBC in pituitary tissue of steers grazing endophyte-infected tall fescue and supplemented with 3 mg Se/d in vitamin-mineral mixes as either sodium selenite (ISe), SEL-PLEX (OSe), or a 1:1 mix of ISe and OSe (MIX) (Chapter 5)

1. Representative of SAS editor programming language using PROC GLM procedure

```
proc means data=SCApituitary MEAN MIN MAX STD STDERR;
class trt;
proc glm data=SCApituitary plots=diagnostics;;
class trt;
model UBC=trt;
lsmeans trt/diff;
run;quit;
```

2. SAS output

3. The SAS System

The MEANS Procedure

Analysis Variable : UBC UBC

trt	N Obs	Mean	Minimum	Maximum	Std Dev	Std Error
B	**7**	23.8142857	23.0600000	24.2800000	0.3923798	0.1483056
G	**7**	23.7642857	23.5150000	23.9350000	0.1665440	0.0629477
R	**6**	23.9116667	23.4200000	24.1400000	0.2583344	0.1054646

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	3	B G R

Number of Observations Read	20
Number of Observations Used	20

The SAS System

The GLM Procedure

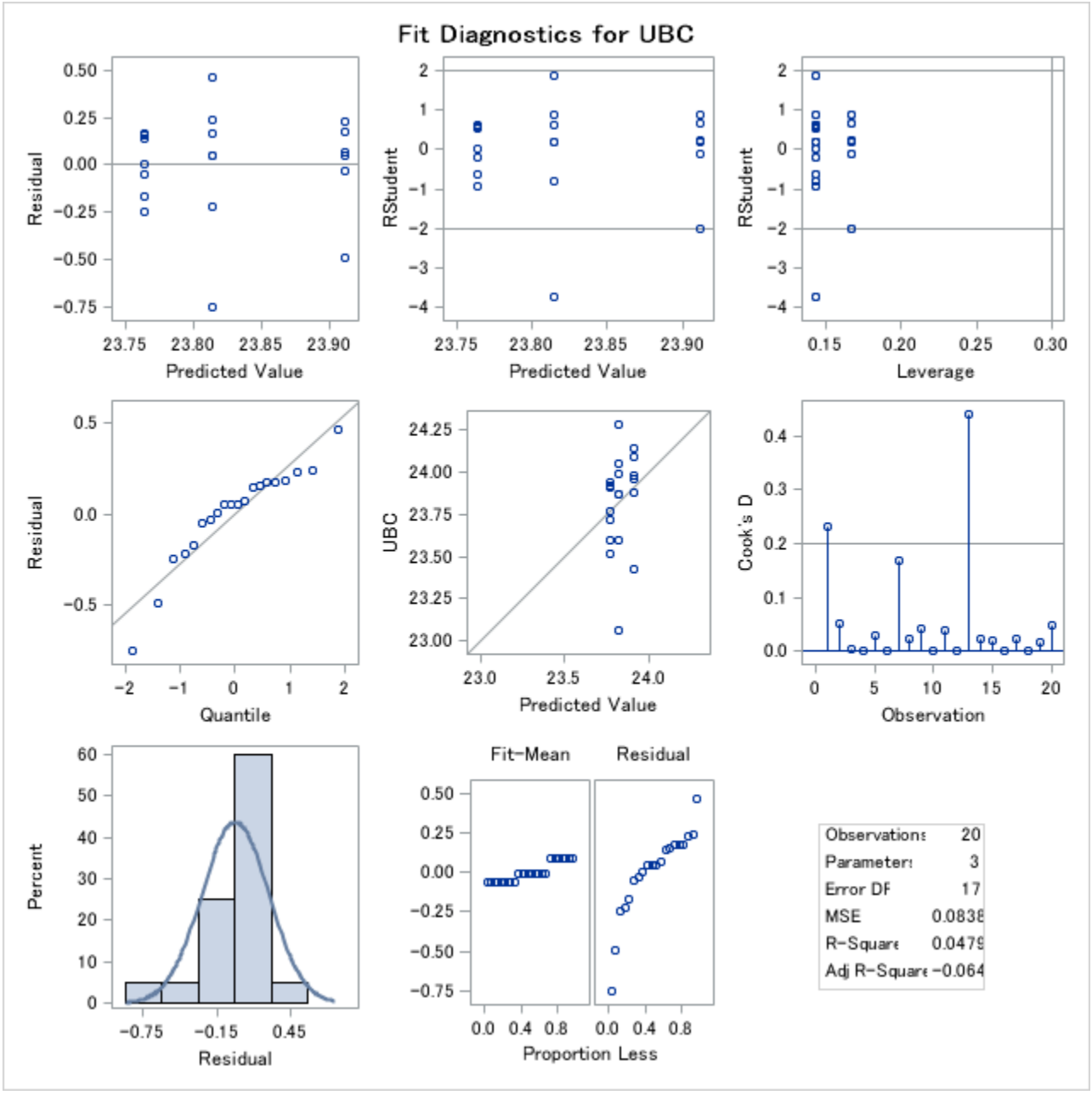
Dependent Variable: UBC UBC

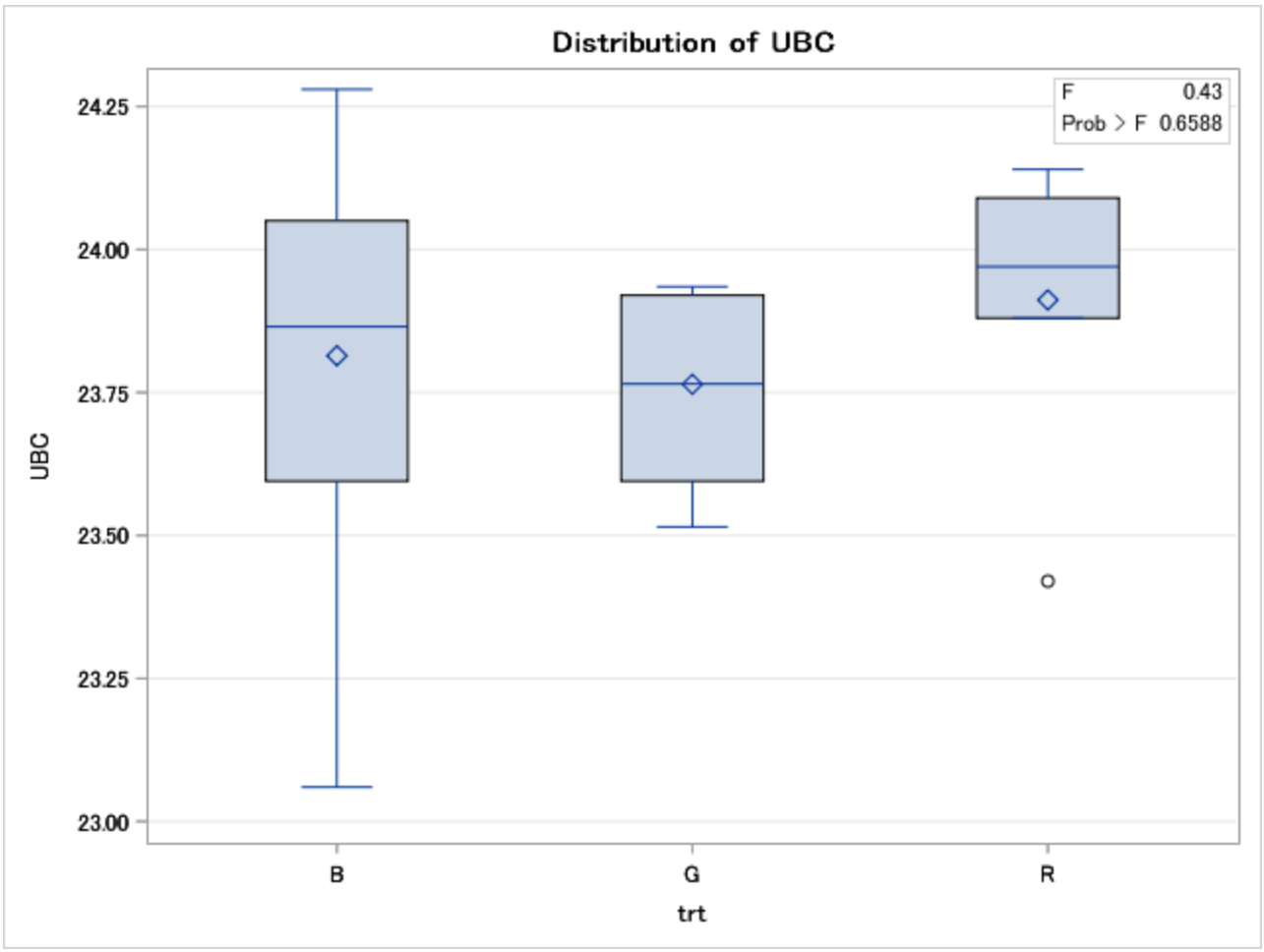
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.07165381	0.03582690	0.43	0.6588
Error	17	1.42387619	0.08375742		
Corrected Total	19	1.49553000			

R-Square	Coeff Var	Root MSE	UBC Mean
0.047912	1.214676	0.289409	23.82600

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	2	0.07165381	0.03582690	0.43	0.6588

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	2	0.07165381	0.03582690	0.43	0.6588




Distribution of UBC
F 0.43
Prob > F 0.6588
24.25
24.00
23.75
23.50
23.25
23.00
UBC
B
G
R
trt

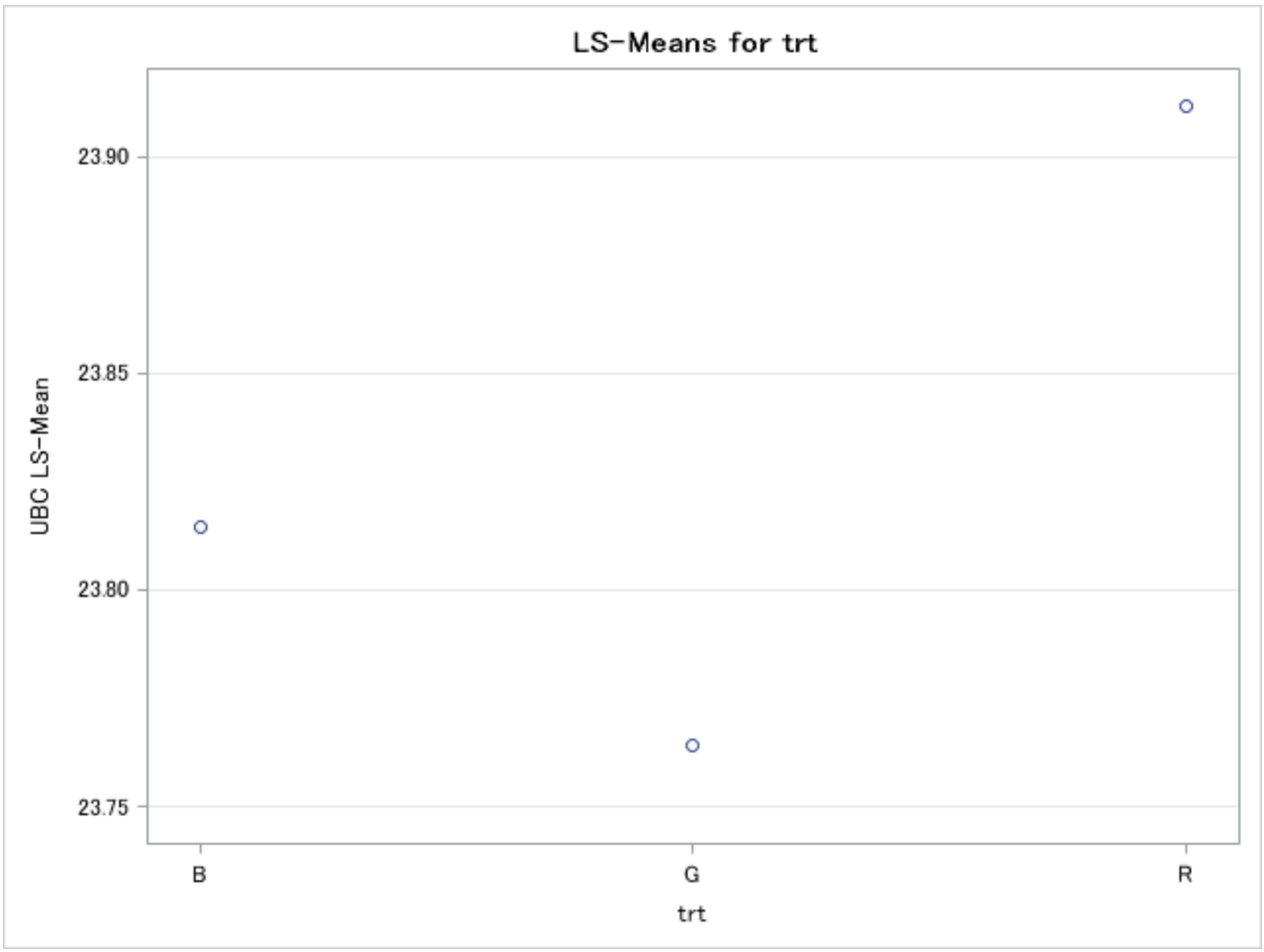
The GLM Procedure

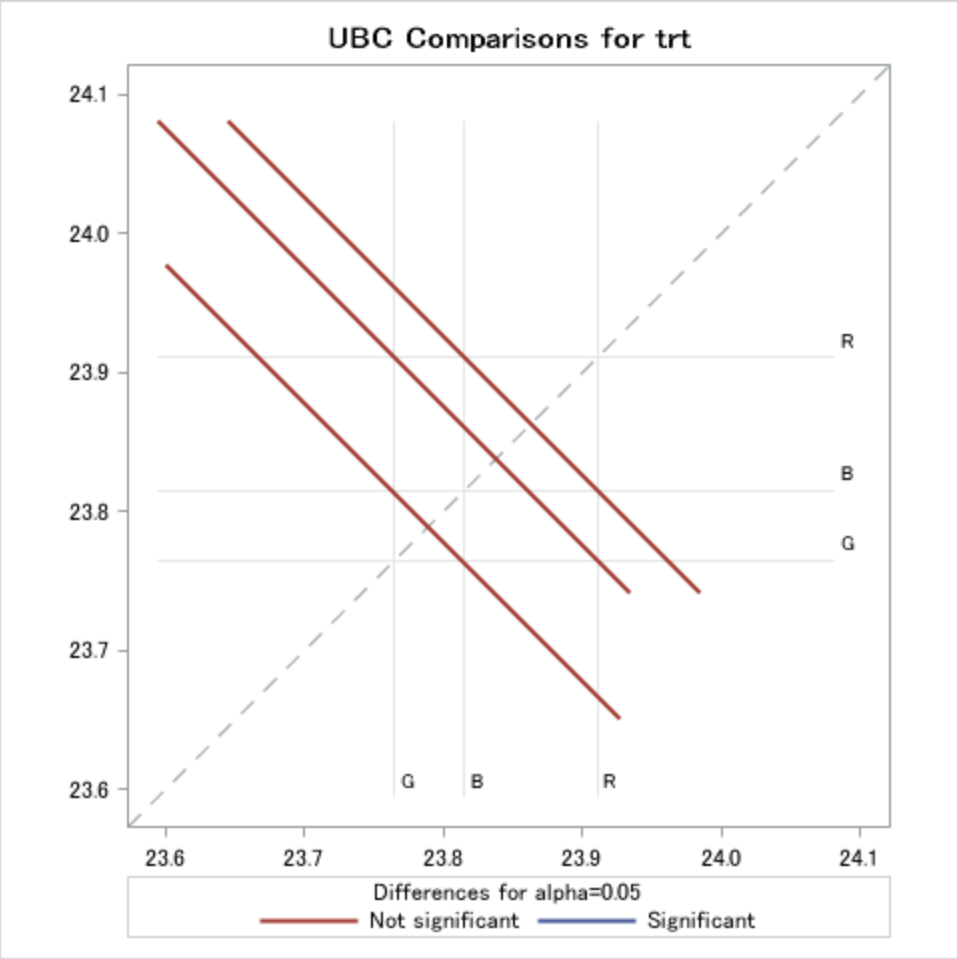
Least Squares Means

trt	UBC LSMEAN	LSMEAN Number
B	23.8142857	1
G	23.7642857	2
R	23.9116667	3

Least Squares Means for effect trt
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: UBC

i/j	1	2	3
1		0.7505	0.5533
2	0.7505		0.3728
3	0.5533	0.3728	


LS-Means for trt
UBC LS-Mean
23.90
23.85
23.80
23.75
B
G
R
trt


UBC Comparisons for trt
24.1
24.0
23.9
23.8
23.7
23.6
R
B
G
G
B
R
23.6
23.7
23.8
23.9
24.0
24.1
Differences for alpha=0.05
Not significant
Significant

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