SELENIUM METABOLISM AND TOXICITY OF INORGANIC AND ORGANIC
SELENIUM SOURCES AND LEVELS ON GROWTH, REPRODUCTION AND
OTHER MINERAL NUTRIENTS IN SWINE

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
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By

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* * * * *

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This dissertation examined the effects of high dietary levels of organic and inorganic Se on growth performance, toxicity characteristic in grower-finisher pigs and the long term effects on sow performance, and the tissue Se concentration and subsequent toxic symptoms in both the sow and progeny. The experiments demonstrated that feeding a diet containing 5 to 10 ppm inorganic Se (sodium selenite) or 15 ppm organic Se (Se-enriched yeast) resulted in toxic responses in grower-finisher pigs. Serum and tissue Se content increased as dietary Se level increased but was higher when organic Se was fed. Colored hair pigs (red or black) were more resistant to Se toxicity compared to white haired pigs in both Se sources. The excretion of the essential micro-minerals (Zn and Mn) increased in the bile as dietary Se level increased and was higher when pigs were fed inorganic Se. When inorganic Se was fed, Se toxicity occurred at 7 ppm, but at 10 ppm organic Se when fed to reproducing sows. Serum GSH-Px activity of sows and pigs was not affected by dietary Se level or source. Serum GOT activities at the 12 wk period increased as dietary Se level increased and were approximately 2 fold higher when pigs were fed inorganic Se. This suggested increased cellular damage as dietary Se level increased particularly when inorganic Se was fed. Dark colored of bile at high dietary Se levels suggested that hepatic tissue damage occurred. Selenium transfer through the placenta and milk from dam to litter was higher when organic Se was provided during gestation and lactation. Tissue and serum Se concentrations of sows and pigs were higher when sows were fed organic Se. This demonstrated that the labile Se (liver) when the organic Se was provided supported high milk Se concentration. The higher Se transfer from dam to litter consequently increased the Se status of progeny when organic Se was fed. Hoof separation and hair loss in nursing pigs at 14
days of age occurred above 7 ppm Se when sows were fed inorganic Se. When organic Se was provided, only hair loss was observed at 10 ppm Se. Hair Se concentration was a good indicator of Se status, particularly when pigs were fed high levels of Se. Amino acid composition of pig hair was similar among three different colored hair (white, red and black) pigs. Red colored hair of gilts contained more Se and thicker than white colored hair when gilts were fed normal diets containing .3 ppm of inorganic Se. Consequently, Se requirement may differ by breed hair color. Hair Se content and hair diameter were affected by hair location of the body.
To My Lord

and My Wife
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"I can do all things through Christ who strengthens me"

- Philippians 4:13 -

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CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

The element selenium (Se) was named by the Swedish chemist J. J. Berzelius in 1818 after its discovery in the residue of sulfuric acid preparations from a mining operation. The early interest in Se was related to its properties as a toxic element. Marco Polo in his travels in western China about the year 1295 A.D. was perhaps the first to describe a disease syndrome resulting from ingestion of seleniferous plants (Rosenfeld and Beath, 1964). He reported a poisonous plant that, if eaten by horses, caused the hooves to drop off (Rosenfeld and Beath, 1964). Loss of hair and nails in humans presumably suffering from chronic selenosis was also described in Colombia by Father Pedro Simon in 1560 (Benavides and Mojica, 1965). Of greater concern, however, was the association of human fetal malformations that was occurring by the consumption of the local foods. In the United States, the toxic role of Se was investigated in certain soils in the Dakotas (Franke, 1934; Moxon, 1937).

Since 1949, three substances had been known to protect rats from a fatal liver necrosis condition (vitamin E, cystine, and Factor 3). Selenium's beneficial role in animal nutrition thus began in 1957 with the finding by Schwarz and Foltz (1957) that a factor in yeast would prevent liver necrosis in rats. It was therefore not until 1957 that Se was discovered to be the active agent of Factor 3. Selenium has since been found to be at the active site of glutathione peroxidase (GSH-Px) (Flohe et al., 1973). Selenium has also been discovered in several
bacterial and other Se-containing enzymes (Arthur et al., 1990; Read et al., 1990). All presently known Se-containing enzymes and proteins contain the selenoamino acid, selenocysteine (Hawkes et al., 1985).

Selenium has an atomic number of 34, and atomic weight of 78.96 in the Vla group of elements of the periodic table which also includes oxygen (O) and sulfur (S). Selenium is thus a semi-metal (or metalloid), which has very similar chemical properties to S. It exists in several allotropic forms including a red powder, red crystals, a dark brown moss, and a silver gray form produced after extensive heating at 200 to 220 °C. Elemental Se (Se) can be further reduced to selenide (Se) or oxidized to selenite (Se) state. Selenium can be volatilized under acidic conditions, and care therefore must be taken to prevent the loss of Se during its analysis. Elemental Se burns in air to form SeO, which emits a characteristic odor resembling rotting horseradish. Elemental Se is not soluble in water, but many of its salts (selenites and selenates) are water soluble (Bennett, 1983).

Selenium is widely distributed in the environment (waters, soil, and air) though generally in very low concentration. (< 1 µg/g). The Se content in limonite rocks sometimes reaches 0.5 mg/g, and 2.6 mg/g in vanadium-uranium rock (Rosenfeld and Beath, 1964). Data on Se-containing rocks are derived from North and South America, Canada, Columbia, Mexico, Australia, New Zealand, Ireland, Bulgaria, Germany, USSR and Mediterranean countries (Rosenfeld and Beath, 1964). Some ground or mountain and waters are reported to contain elevated concentrations of Se (e.g., Colorado channels or subterranean waters in the region Orsk). Selenium and its compounds have found broad technological applications; these include the chemical industry (catalyst), electronics (for production of semiconductors, photocells, and rectifiers), machine industry (for obtaining high-grade steel), glass industry (for staining or coloring of glass), rubber industry (for acceleration of vulcanization), and various pharmaceutics (veterinary Se preparations in treatment of Se deficiency). In agriculture, the organoselenium compounds are used as bactericides, fungicide, and herbicides (Bem, 1981).
1.2 FUNCTIONS OF SELENIUM IN ANIMAL

1.2.1 Body Selenoprotein(s)

Under physiological conditions Se has several metabolic fates. A major one in a quantitative sense is its incorporation into various selenoproteins. Selenite labeled \(^{75}\)Se was administered in the drinking water to rats fed a Se-deficient diet, and revealed that over 80\% of the \(^{75}\)Se in the rat was as selenocysteine (Hawkes et al., 1985).

Methylated forms of Se are produced for excretion (Bopp et al., 1982), and Se has been shown to be incorporated into certain tRNAs in cultured cells (Ching, 1984). The physiological significance of these tRNAs has not been completely established. The selenoproteins with known enzymatic activity are generally redox enzymes containing various selenoproteins in their active sites. Replacing Se with sulfur reduces its activities (Axley et al., 1991; Berry et al., 1991; Rocher et al., 1992), demonstrating that these enzymes depend upon Se.

This literature review will focus on proteins containing Se as selenocysteine and will refer to them collectively as selenoproteins.

1.2.1.1 Selenocysteine

All proteins that incorporate Se stoichiometrically contain it in the form of selenocysteine. This was first identified in protein A of the glycine reductase complex of Clostridium sticklandii by Cone et al. (1976). It has since been found in several other proteins, and a specific mechanism for its synthesis and incorporation has been characterized. Selenocysteine is identified as the only seleno-amino acid which is present in intracellular selenoproteins (Calomme et al., 1995a).

1.2.1.2 Selenomethionine

In Se accumulator plants Se is incorporated into the non-proteinaceous amino acids and analogs such as Se-methylselenocysteine, seleno-cystathione, selenocystine, and
selenohomocysteine (Trelease and Beath, 1949; Shibata et al., 1992). These plants as well as
the soil microflora can volatilize Se compounds. The offensive odor from Se accumulator
plants and microflora, such as Penicillium, Acremonium, Ulocladium, or Fusarium, is likely
dimethylidiselenide, with lesser amounts of dimethylselenide (Frankenberger and Karlson, 1992).
Nonaccumulator plants such as grains and grasses also produce dimethylselenide if moderate
levels of soil Se are present. The major Se component of these nonaccumulator plants appears
to be the proteinaceous amino acid selenomethionine (Olson et al., 1970).

Plant and bacteria synthesize Se-containing amino acids, including selenomethionine. Most
evidence suggests that animals do not distinguish this amino acid from methionine
Because selenomethionine can substitute for methionine during protein synthesis in rat
(McConnell and Hoffman, 1972). Thus it appears to be incorporated into various body protein in
place of methionine and appear to have no Se-related function. The incorporation of Se into
animal proteins when administered as selenomethionine is directly correlated with the quantity of
selenomethionine consumption and is inversely correlated with methionine intake (Waschulewski
and Sunde, 1988).

The Se from selenomethionine is hydrolyzed and utilized in specific Se pathways when the
protein is catabolized. Selenomethionine can be metabolized to selenocysteine by the
transsulfuration pathway. However, the produced selenocysteine is catabolized by
selenocysteine β-lyase (Esaki et al., 1981) and does not serve as a form of selenocysteine can
be incorporated into protein in place of cysteine by some bacteria (Ganter, 1986; Stadtman et
al., 1989). Such incorporation should be prevented by selenocysteine β-lyase activity in cells
containing this enzyme.

1.2.1.3 Type I Iodothyronine 5'-deiodinase (5'DI)

Thyroid function depends on the conversion of the various thyroid iodothyroxine compounds to
triiodothyronine (T3). Several deiodinase enzymes are involved in there conversions but one of
them is a selenoprotein containing enzyme (Arthur et al., 1990; Behne et al., 1990; Berry et al.,
The enzyme is a homodimer and each 27-kDa subunit contains one selenocysteine (Berry et al., 1991). It is present in the endoplasmic reticulum of liver and kidney (Berry and Larsen, 1992). This enzyme converts the prohormone thyroxine (T₄ or 3,5,3',5'-tetraiodothyronine) to the biologically active hormone triiodothyronine (T₃ or 3,5,3'-triiodothyronine), and the metabolic inactive reverse triiodothyronine (rT₃ or 3,3',5'-triiodothyronine) in diiodothyronine (3,3'-di-T₂) (Calomme et al., 1995b). A reducing substrate is required which, in vivo, is probably glutathione (Burk and Hill, 1993). In Se deficiency, thyroxine (T₄) levels in plasma rise as a consequence of the decrease in 5'DI activity (Beckett et al., 1987). Triiodothyronine levels are thus depressed slightly by the Se deficiency (Beckett et al., 1987), but Se-deficient animals remain euthyroid (Burk and Hill, 1993).

1.2.1.4 Selenoprotein P (Se-P)

The existence of a plasma selenoprotein other than GSH-Px has been known for over 15 years, but the purification and characterization of Se-P has been demonstrated only during the last five years. In the rat, 65% of plasma Se is Se-P (Read et al., 1990). A cDNA for Se-P has been cloned and sequenced (Hill et al., 1991). There are ten TGAs (UGAs in mRNA) in the Open Reading Frame (ORF), indicating that the protein contains ten selenoprotein residues in its primary structure. It is the only selenoprotein characterized thus that contains more than one selenocysteine per polypeptide chain. The protein is a glycosylated single polypeptide chain of 41 kDa (Read et al., 1990). The function of Se-P is presently not known. Its appearance in plasma correlates highly with protection against free radical injury of the liver (Burk et al., 1980; Burk, 1991), and thus, Se may be a free radical scavenger. Another hypothesis is that it transports Se from the liver to other tissues, suggesting that it is synthesized in sites other than the liver (Hill et al., 1992). The half-life of Se-P is apparently not affected by the Se status of the animal (Burk et al., 1991). These observations make a transport role for Se-P unlikely.
1.2.2 Glutathione Peroxidase (GSH-Px)

Studies of Se-dependent protection against rat red blood cell hemolysis was conducted in the laboratory of W. G. Hoekstra and culminated in 1973 with the discovery that rat red cell GSH-Px is a selenoenzyme (Rotruck et al., 1973). Shortly thereafter, Flohe et al. (1973) reported that bovine blood GSH-Px contained one Se atom per subunit. Since then, this enzyme has been purified from a number of sources including human tissues, and has shown to contain Se in each case. It has a wide distribution in animal cells and is present in the mitochondria cytosol (Burk, 1983). Nutritional deficiency of Se results in a decline in tissue Se-dependent GSH-Px activity. Rat liver activity falls to undetectable levels after only 4 weeks of a Se-deficient dietary regimen (Hafeman et al., 1974). Other tissues experience a more gradual decline in GSH-Px activity. These observations indicate that a major biochemical effect of Se is to maintain GSH-Px activity.

The understanding of Se-dependent GSH-Px was complicated in 1976 by the discovery of a non Se-dependent GSH-Px activity (Lawrence and Burk, 1976). The glutathione S-transferases account for the non-Se-dependent GSH-Px activity (Prohaska and Ganther, 1977). These enzymes are found in fewer cell types than Se-dependent GSH-Px. In rat liver, glutathione S-transferase is present in the cytosol, mitochondria, and micorsomes. Their GSH-Px activity in the microsomes appears to be lower than that in the other sites (Burk et al., 1982). Rat liver glutathione S-transferase activity is 50 to 100% higher in Se-deficient male rat liver cytosol than in the control and presumably also non-Se-dependent GSH-Px activity (Lawrence et al., 1978). The reason for this increase is unknown, but it may compensate for losses of Se-dependent GSH-Px. Therefore, these enzymes should be increased during a Se deficiency. Observations from studies with rats (Hill et al., 1987; Lawrence et al., 1978) are consistent with this hypothesis. Whereas, Se-deficient birds (Xu and Diplock, 1983; Kim and Combs, 1992) and fish (Bell et al., 1986) with severely reduced tissue GSH-Px activities have not shown increased glutathione S-transferase activities. Kim and Combs (1992) demonstrated that the increase in
glutathione S-transferase activity did not occur in Se-deficient chicks unless they were also deficient in vitamin E.

Lawrence and Burk (1978) reported the presence of both Se-dependent and Se-independent GSH-Px activities in pig livers. Approximately 67% of the total liver GSH-Px activity of the pig was Se-independent, whereas the data of Meyer et al. (1981) suggested that 45 to 60% was Se-independent. The amount of Se-independent GSH-Px activity was fairly consistent at the different dietary Se levels, the relative percentage of Se-independent activity was greatest for those fed .1 ppm Se because of the lower Se-dependent GSH-Px activity (Meyer et al., 1981). These results suggest that the increase in total liver GSH-Px activity largely reflected the increase of the Se-dependent form of the enzyme, as there was no further increase in activity at higher dietary Se levels.

The glutathione S-transferases catalyze the conjugation of electrophilic compounds and metabolites with glutathione, thereby constituting one of the major detoxification mechanisms in the liver. Some of these enzymes have nonSe-dependent GSH-Px activity.

Although the Se-dependent and non Se-dependent GSH-Px carry out the same general reaction, many of their characteristics differ (Burk and Lawrence, 1978). Selenium-dependent GSH-Px destroys both H₂O₂ and organic hydroperoxides. Non Se-dependent GSH-Px does not metabolize H₂O₂ and it accumulates (Lawrence and Burk, 1978). The enzyme also has higher apparent Kms toward organic hydroperoxide substrates than does Se-dependent glutathione peroxidase.

The major physiological function of Se dependent GSH-Px is thought to maintain low levels of H₂O₂ and other hydroperoxides in the cell to prevent tissues from peroxidation damage. Accumulation of these reactive substances could lead to impaired function or destruction of the cell. Experiments in which H₂O₂ or organic hydroperoxides were added to isolated cells or perfused livers demonstrated that the GSH-Px function in intact cells (Sies et al., 1972; Eklow et al., 1981). The H₂O₂ concentration in the cell may be regulated by Se-dependent GSH-Px acting in concert with catalase (Jones et al., 1981).
Table 1 summarizes some of the properties of these activities (Burk, 1983).

<table>
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<th>( \text{H}_2\text{O}_2 )</th>
<th>Organic Hydroperoxides</th>
<th>Effect of Se Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenium-dependent</td>
<td></td>
<td>+</td>
<td>+</td>
<td>↓↓↓↓</td>
</tr>
<tr>
<td>Glutathione Peroxidase</td>
<td>Seleno-glutathione</td>
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<tr>
<td></td>
<td>peroxidase</td>
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<tr>
<td>Nonseelenium-dependent</td>
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<tr>
<td>Glutathione</td>
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</tbody>
</table>

Table 1. Comparison of rat liver Se-dependent and nonSe-dependent glutathione peroxidase (Burk, 1983)

The role of the GSH-Px in the metabolism of the organic hydroperoxides is not clear. Fatty acid hydroperoxides should be the major organic hydroperoxide form in the cell. They serve as substrates for both GSH-Px when present in the unesterified form (Pierce and Tappel, 1978), but may not be available to the enzymes when esterified in phospholipids (McCay et al., 1976), which are the likely form found in the cell. Consequently, it cannot be stated with certainty that the GSH-Px metabolize fatty acid hydroperoxides in the cell. Sies and Moss (1978) suggests that Se dependent GSH-Px may help regulate mitochondrial substrate oxidation. Oxidation of pyruvate was shown to decrease when hydroperoxide was being metabolized by Se-dependent GSH-Px but no decrease was found when Se deficient mitochondria were used. This indicates that the selenoenzyme may have more subtle functions than the destruction of hydroperoxides.

Until recently, researchers knew little of the effect of Se deficiency on glutathione metabolism. Liver glutathione concentration is measured by determining the rate of synthesis of the compound in the liver and the rate of glutathione release into the bile and blood (Bartoli and Sies, 1978). Experiments with isolated hepatocytes and perfused livers indicate that Se
deficiency markedly accelerates glutathione synthesis in rat liver (Hill and Burk, 1982) which is balanced by an increased glutathione concentration into blood. There appears to be no effect of Se deficiency on the release of glutathione into the bile. Liver cysteine concentration is lowered in the Se-deficient rat (Hill and Burk, 1982), presumably because cysteine is used in the synthesis of glutathione. Glutathione concentration in blood plasma is two to three times higher in Se-deficient rats than in the controls as a result of the increased release of the compound into the blood. At present it is not known why Se deficiency increases hepatic glutathione turnover. The increase may be in compensation for the loss of plasma GSH-Px activity. Alternatively, glutathione could be lost from the liver cell as a consequence of faulty regulation of its release. Little is known about the mechanism and regulation of glutathione release into the blood (Burk, 1983).

Glutathione peroxidase activity in certain tissue (kidney, heart) may therefore be a more accurate indicator of Se adequacy than is Se content of the tissue (Chow and Tappel, 1974). Thompson and Fraser (1983) suggested that erythrocyte GSH-Px would be the most sensitive indicator particularly at lower levels of Se uptake. However, GSH-Px does not appear to be a sensitive index of excessive dietary Se intake (Goehring et al., 1984b). Blood GSH-Px data demonstrate that excess sodium selenite elevates GSH-Px to a level that is possibly greater than the animal's physiological requirement of this enzyme. The plateauing of GSH-Px activity while serum Se concentration continued to rise suggests that the Se requirement had been met.

In dietary liver necrosis, a clear-cut relation between GSH-Px levels in the liver and the development of liver necrosis has been established. During the latent phase of dietary liver necrosis, i.e., 6 to 8 days before death, the GSH-Px activity in livers are reduced by 80 to 90%. A single injection of selenite (50 μg Se/100 g of body weight) is sufficient to reestablish normal enzyme levels within 1 to 2 days. The 6 to 8 day time lag indicates that Se is not simply incorporated into a preexisting apoenzyme, but that the enzyme is synthesized de novo (Schwarz, 1976).
Four different glutathione peroxidases were discovered since 1973.

1) Cellular GSH-Px (GSH-Px) : This enzyme was discovered as an erythrocyte enzyme that protects against oxidative hemolysis (Mills, 1957) and later found to be Se-dependent (Rotruck et al., 1973). For many years, cellular GSH-Px represented the only known biochemically functional form of body Se. Cellular GSH-Px is present abundantly in erythrocytes, kidney, and liver (Chambers and Harrison, 1988).

2) Phospholipid hydroperoxide GSH-Px (phGSH-Px) : This has been purified from pig heart (Ursini, 1985). It is a monomer of 20 kDa and is similar to one subunit of the other GSH-Px forms. It contains one selenocysteine. Thiol compounds other than glutathione can serve as its reducing substrate. This enzyme catalyzes the reduction of phospholipid hydroperoxide, cholesterol hydroperoxide, and linoleic acid hydroperoxide much more effectively than the reduction of H₂O₂ and tert-butyl hydroperoxide (Chu et al., 1993). phGSH-Px is found in several tissues but has a distribution different from that of cGSH-Px. Relatively little is present in rat liver while it is quite abundant in the testis (Roveri et al., 1992). It has been suggested that this enzyme plays a role in eicosanoid metabolism (Ursini et al., 1987) and that it protects against lipid peroxidation (Ursini and Bindoli, 1987).

3) Plasma or extracellular GSH-Px (eGSH-Px) : Plasma GSH-Px was recognized as a different enzyme from cGSH-Px (Takahashi and Cohen, 1986). Since then, its presence has been demonstrated in milk, and it has been referred to as eGSH-Px. Its activity in plasma is a convenient index of Se nutritional status. Plasma GSH-Px shares some sequence identity with cGSH-Px but is clearly a separate gene product (Takahashi et al., 1990). It consists of four identical 23 kDa subunits, each of which contains one selenocysteine. Recent work indicates that it is synthesized in the kidney and in the lung (Chu et al., 1992). The function of eGSH-Px is not known. The fact that its reducing substrate, glutathione, is present at very low concentrations in
extracellular fluids has led to suggestions that the enzyme might have a function other than as a GSH-Px.

4) Gastrointestinal GSH-Px (GSH-Px-GI) : This enzyme is localized in cytosol (Chu et al., 1993). The chemical function of this enzyme is similar with cellular GSH-Px consequently, it catalyzes the reduction of H₂O₂, tert-butyl hydroperoxide, cumene hydroperoxide, and linoleic acid hydroperoxide with glutathione, but not of phosphatidylcholine hydroperoxide. GSH-Px-GI mRNA was readily detected in human liver and colon and occasionally in human breast, but not other human tissues including kidney, heart, lung, placenta, or uterus. In rodent tissues, GSH-Px-GI mRNA is only detected in the gastrointestinal tract, and not in other tissues including liver. In fact, GSH-Px-GI appears to be the major glutathione-dependent peroxidase activity in rodent GI tract. The GSH-Px-GI could play a major role in protecting mammals from the toxicity of ingested lipid hydroperoxides (Chu et al., 1993).

Glutathione peroxidase is not the only enzyme affected in Se deficient pigs. Pigs fed low Se diets had elevated serum glutamic oxaloacetic transaminase (sGOT), serum glutamic-pyruvic transaminase (sGPT) and serum lactic acid dehydrogenase (LDH) activities, reflecting oxidative damage to cells which results in their subsequent release into the circulatory system. Selenium supplementation prevented increases in sGOT and sGPT (Ewan and Wastell, 1970). In addition, supplemental Se decreased the elevation of LDH activity (Ewan and Wastell, 1970).
1.2.3 Inorganic and organic selenium

1.2.3.1 Inorganic Se

Alexander and Whanger (1983) showed that at equal Se intakes by rats, selenite produced higher GSH-Px in all tissues than did the Se from raw, cooked, or canned-tuna Se. The supplementation of sodium selenite (200 μg/d for 8 wk) resulted in 118% increase in cytotoxic lymphocyte-mediated tumor cytotoxicity and 82.3% increase in natural killer cell activity when compared to baseline values (Kiremidjian-Schumacher et al., 1994). This apparently was related to the ability of the nutrient to enhance the expression of receptors for the growth regulatory lymphokine interleukin-2, and consequently, the rate of cell proliferation and differentiation into cytotoxic cells. This indicates that the immunoenhancing effects of Se in humans require Se supplementation above the repition levels produced by normal dietary Se intakes.

It has been reported that both sodium salt forms of Se (i.e., selenite and selenate) are of potential danger to humans because of their high water solubility when in direct contact with skin and mucous membranes; hence, toxic responses might occur (Echevarria et al., 1988b). Calcium selenite is also considered toxic if it is inhaled or swallowed or when it comes into contact with skin, but its solubility is less rapid than that of sodium selenite and thus presents less immediate danger to humans. Mahan and Magee (1991) reported that calcium selenite responded similarly as sodium selenite when tissue Se concentrations and serum GSH-Px activities of weanling swine were fed at approved, marginally toxic, and toxic dietary Se levels. Selenium bioavailabilities in young chicks and lambs were similar when calcium or sodium selenite have been fed during both short (< 10 d) and long (40 or 80 d) time periods (Echevarria et al., 1988a; Henry et al., 1988).
1.2.3.2 Organic Se

The major Se component of nonaccumulator plants such as grains and grasses, appears to be the proteinaceous amino acid selenomethionine (Frankenberger and Karlson, 1992). The organic Se found in feed ingredients is comprised of several organic forms but selenomethionine represents about 50% of the Se in cereal grains (Olson and Palmer, 1976). However, in Se accumulator plants, Astragalus, Se is incorporated into nonproteinaceous amino acids such as Se-methylseleno cysteine, selenocystathione, selenocystine, and selenohomocysteine (Trelease and Beath, 1949; Shibata et al., 1992).

Gabrielsen and Opstvedt (1980) reported that selenomethionine had 78% of the availability when compared to sodium selenite. This was followed by fish meals (48% for capelin and 34% for mackerel), corn gluten meal (26%), and soybean meal (18%). The relatively low availability reported for fishmeal Se might be related to the lower level of antioxidants in the diets (Cantor et al., 1975a, 1975b) or the higher concentration of heavy metals.

An enriched Se-yeast product has been produced by feeding a yeast strain with a high S requirement with sources of inorganic and organic Se (Mahan, 1995). Because S and Se are chemically similar, Se is incorporated into yeast cell protein structures by replacing the S with Se. Kelly and Power (1995) demonstrated that approximately 94% of the Se in the Se-enriched yeast source was organically incorporated into one of several seleno-amino acid analogs, the major one being selenomethionine. Bird et al. (1997), however, reported that selenomethionine accounted for no more than 20% of all Se-containing materials in selenized yeast. In addition to selenomethionine, the other compounds that had been identified included selenocysteine, Se-methylselenocysteine and selenoethionine (representing ~20%). On top of that, there were several unidentified peaks that combined to represent 40-50% of the total when more sophisticated analytical method, high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) (Bird et al., 1997).
The dietary supplementation with the Se-enriched yeast product resulted in the increase retention of Se in all tissues, and changes in distribution and retention of Cu, Zn, Mn, and Fe in rat tissues (Djuric et al., 1995).

1.2.4 Interactions of selenium with other elements

There are numerous interactions of Se and other chemical substances (sulfates, phosphates, mercury (Hg), arsenic (As), cadmium (Cd) and other metal) which can affect their distribution, retention and toxicity in the body (Diplock, 1976). The interactions are complex and not yet fully understood. Lowry and Baker (1986) revealed that among the various As compounds examined As₂O₃ was most protective, followed by phenylarsine oxide (trivalent organic), As₂O₅, phenylarsonic acid, roxarsone and arsanilic acid (the latter three being pentavalent organic sources). Oral administration of L-cysteine stimulates growth of selenite-intoxicated chicks, but not those fed toxic levels of selenomethionine and L-cysteine. Cysteine and roxarsone were found to be synergistic in ameliorating the growth depression caused by 15 mg Se/kg diet provided from Na₂SeO₃ (Baker and Czannecki-Maulden, 1987).

Levander and Morris (1970) demonstrated that methionine was generally more efficacious than cysteine in reversing the growth depressions caused by selenate toxicity in rats. The authors speculated that methionine, via S-adenosyl methionine formation, may have facilitated the synthesis of methylated selenide compounds for excretion via urine and expired air.

Selenite or selenate, administered to rats orally or via injection reduced the acute or chronic toxicity of Hg and methyl mercuric ions by redistribution of tissue Hg rather than by an increase in excretion of the element (Parizek et al., 1974). Dietary Se at 5 ppm protected rats against toxicity of acute lethal doses of methyl and mercuric mercury (Potter and Matrone, 1974). Selenite increased the percentage of Hg retained in liver and spleen and decreased that in kidney compared with animals untreated with Se but there was no subsequent on hair Hg. It has been postulated that cystine or the thiols

1) provided protein-sulfur binding sites for Hg,
2) that Se catalyzed mercury to a less damaging form, or
3) that Se reacted directly with Hg and caused its excretion.

Supplementation of vitamin E can protect Japanese quail against methyl mercury toxicity consequently, vitamin E and Se level of .05 to .1 ppm Se are recommended (Welsh and Soares, 1975). Supplemental Hg has reduced the transfer of Se in rats from dams to fetus and milk. Injected methylated Se compounds with mercuric ion, however, were much more toxic for rats than when either form of metal alone (Ammerman et al., 1977).

Kezhou et al. (1987) reported that the Cu\(^{2+}\) ion is much more effective in ameliorating the effects of selenite toxicity in rats than the SO\(_4\)^{2-} ion. Selenite caused the increase of the hepatic concentration of Cu, and that the body accumulation of Se was a greater factor in the mortality from selenite toxicity among rats than the protein-calorie malnutrition associated with selenite toxicity (Kezhou et al., 1987). Ahmed et al. (1988) reported that Se metabolism in the rat, absorption and distribution of Se in body tissue, was not affected by at normal dietary concentrations of Cu, Fe, Cd, Mo, Mn, especially if adequate amounts of Se are being consumed. Underwood (1977) suggested that the feeding of high protein or high sulfate containing diets alleviated Se toxicity.

It was noted that vitamin C levels were lowered under Se poisoning condition and that the levels of selenides could be reduced by addition of vitamin C (Svirbely, 1938). It has also been suggested that vitamin C resulted in a decreased rate of Se excretion and increased the toxicity of Se compounds (Amdur, 1958).

High levels of Se-enriched yeast in diet caused decrease of Fe content in liver, kidney, and heart muscle but increased its content in brain, blood, and spleen of rat tissue (Djujic et al., 1995). Zinc content in tissues also decreased by Se supplementation in spleen, muscle, hair except the pituitary gland. Manganese content was decreased in the liver, spleen, and heart but it was increased in brain and hair when rats were fed high levels of Se-enriched yeast diet.

Copper content also decreased in liver, spleen, and muscle but was increased in heart and brain.
Interaction between Zn and Fe, which is probably and indirect effect via Cu because Zn is antagonist to Cu absorption, while Cu is essential for normal Fe metabolism (Djuić et al., 1995).

1.2.5 Relationship with vitamin E

Selenium is an essential constituent of GSH-Px, with 4 g-atoms of Se per mole of enzyme (Rotruck et al., 1973). Approximately 40% of whole-body Se in the rat is GSH-Px when selenite is the dietary Se source (Burk, 1991). Among other functions, this enzyme aids in protecting cellular and subcellular membranes from oxidative damage (Sies and Moss, 1978). Vitamin E is a specific lipid-soluble antioxidant in the cellular membrane, while Se functions as a component of GSH-Px that destroys peroxides in the cytosol before they can attack cellular membranes. If lipid hydroperoxides are allowed to form in the absence of adequate tocopherols and GSH-Px, direct cellular tissue damage can result.

Peroxidation of lipids can destroy the structural integrity of the cell and cause metabolic derangement. Vitamin E in the cellular and subcellular membranes is the first line of defense against peroxidation of vital phospholipids. Even with adequate vitamin E, however, some peroxides are formed. Selenium, as part of the enzyme GSH-Px, is a second line of defense, destroying these peroxides before they have an opportunity to cause membrane damage. Vitamin E, Se, and S-containing amino acids, through different biochemical mechanisms, prevent some of the same nutritional diseases. Vitamin E prevents fatty acid hydroperoxide formation, S amino acids are precursors of GSH-Px, and Se is a component of GSH-Px (Smith et al., 1974).

Scott et al. (1982) have summarized the sparing effect of Se and vitamin E on each other. Selenium spares vitamin E in at least three ways:

1. preserves the integrity of the pancreas, which allows normal fat digestion, and thus normal vitamin E absorption

2. reduces the amount of vitamin E required to maintain integrity of lipid membranes via GSH-Px

16
(3) aids in some unknown way in the retention of vitamin E in the blood plasma

Vitamin E also reduces the Se requirement in at least two ways:

(1) maintains body Se in an active form, or prevents its loss from the body

(2) prevents destruction of membrane lipids within the membrane

The latter thus inhibits the production of hydroperoxides, and reducing the amount of the Se-dependent enzyme needed to destroy peroxides formed in the cell. Vitamin E and Se are mutually replaceable, to some degree, but there are limits below which substitution is ineffective. In a severe Se deficiency, vitamin E does not prevent or cure exudative diathesis, while as little as .05 ppm Se completely prevented this disease (Scott, 1980).

Clearly there exists several similar nutritional and biochemical relationships between Se and vitamin E. In the most straightforward relationship, both nutrients are antioxidants and are therefore related in function (Hoekstra, 1975). Observations by several groups have indicated that Se deficiency can affect \( \alpha \)-tocopherol metabolism. Two research groups which have studied vitamin E-deficient rats reported that a low plasma vitamin E level was increased significantly by the simultaneous induction of Se deficiency (Burk et al., 1982; Cheeke and Oldfield, 1969). These workers suggested that Se influenced the distribution of vitamin E in the body when the animal was deficient. Fisher and Whanger (1977) showed a greater rate of tritium excretion in the urine of Se-deficient rats after administration of tritiated \( \alpha \)-tocopherol. They concluded that vitamin E is metabolized more rapidly in Se-deficient rats. Hills and Burk (1984) also reported that Se deficiency in the rat depressed hepatic microsomal \( \alpha \)-tocopherol content. Thus, Se deficiency appeared to increase the loss of \( \alpha \)-tocopherol from the body. The cause of this loss has not been established but most likely is related to increased \( \alpha \)-tocopherol oxidation under conditions where Se-dependent antioxidant mechanism are impaired.

Oxidative stress results from an imbalance between the production of oxygen-centered free radicals and their safe disposal (Sies, 1985). Free radicals, also referred to as Reactive Oxygen
Metabolites (ROM) are the products of normal metabolism but produce no detrimental effects on physiological functions when a balance exists between the production and safe disposal of ROM in maintained cells (Halliwell, 1987). However, a deficiency of the natural protective substances or excess exposure to substances that can stimulate ROM production may impair animal health and performance. Vitamin E, is the body's primary lipid-soluble antioxidant, and Se, is a necessary component of GSH-Px, both critical for the defense against ROM. Brezinska-Slebodzinska et al. (1994) suggested that inadequate dietary antioxidants may increase oxidative stress, production of lipid peroxides, and increase the incidence of retained membranes in dairy cows.

It appears that the incidence of Se-responsive diseases in livestock, particularly white muscle disease, is generally higher in areas where the Se content of the crops is low. There are exceptions, where, in some areas the incidence of such diseases is low even though the forages have low Se content (i.e., < 0.05 ppm). The researchers believe that high levels of vitamin E in forage may compensate for the Se deficiency in these areas (Beauchamp et al., 1969; Lannek and Lindberg, 1975). With few exceptions, white muscle disease outbreaks occur on pasture containing ≤ 0.05 ppm Se. In some outbreaks, however, there appears to be an association with a concurrent vitamin E deficiency (Lannek and Lindberg, 1975).

1.2.6 Semen quality

It has been reported that Se is incorporated into the developing spermatozoa of mice and rats (Gould, 1970; Gunn and Gould, 1970; Brown and Burk, 1973). However, Se incorporation seems to occur at an early stage of development, such as late spermatocyte or early spermatid (Gould, 1970). Although a great amount of Se is taken up by interstitial testicular tissue (Gunn and Gould, 1970), the localization of Se in the midpiece of sperm could indicate a specific role of Se in the mitochondria (Brown and Burk, 1973).

Selenium deficiency is responsible for testicular and sperm damage. Although Se deficient male rats exhibited active spermatogenesis in the majority of seminiferous tubules, the
spermatozoa number was lower than in Se supplemented controls. Similarly, Se deficient male rats had active spermatogenesis in only some seminiferous tubules and spermatozoa were nonmotile (Wu et al., 1973). More than 90% of the spermatozoa had morphological abnormalities, particularly breakage of the midpiece. Sperm abnormalities and low motility observed in Se deficient animals have not been prevented or corrected with antioxidant supplementation, including vitamin E (Wu et al., 1973).

The biological reason for the accumulation of Se in the midpiece of sperm has been investigated. The outer membrane of the mammalian sperm mitochondria is rich in cystine and contains most of the selenoprotein of the sperm in rats (Calvin and Cooper, 1979), and bulls (Pallini and Bacci, 1979). This specific Se-protein forms a fibrous fraction of the sperm tail which weighs 17,000 daltons in the rat and has no GSH-Px activity (Calvin, 1978).

Selenium plays an important role in the formation of the SDS-insoluble shells or ghosts (Pallini and Bacci, 1979). The protein that binds Se in the sperm mitochondria of bulls has a molecular weight in the range of 10,000 to 20,000 daltons (Pallini and Bacci, 1979). This protein has a molecular weight similar to one subunit of GSH-Px, but its relative abundance in spermatozoa does not agree with the low levels of the enzyme (Brown et al., 1977). Consequently, Se in bull spermatozoa may have two main functions:

1) structural Se binds to a protein in the developing spermatozoa, and
2) a constituent of GSH-Px (Smith et al., 1979; Senger, 1980).

The role of Se in boar reproduction is currently controversial. Liu et al. (1982) demonstrated that Se deficient boars had a lower concentration of epididymal spermatozoa than those supplemented with Se, and had a much higher incidence of proximal cytoplasmic droplets. This is in contrast to other experiments in which the addition of inorganic Se to the diets of rams, boars, and dairy or beef bulls did not improve their semen quality (Buchanan-Smith et al., 1969; Smith et al., 1979; Bartle et al., 1980; Segerson and Johnson, 1980; Segerson et al., 1981; Henson et al., 1983). It is possible that the animals in these latter trials were not deficient in Se and their reproductive products had not been affected (Marin-Guzman et al., 1997). Boars fed
low-Se diets produce a higher percentage of abnormal sperm, mainly by disrupting tail morphology. Boars with a low Se status had fewer sperm reaching and penetrating the zona pellucida, and this could affect ovum fertilization rate (Marin-Guzman et al., 1997).

Mammalian spermatozoa are harmed by H₂O₂ and lipid peroxidation (Alvarez and Storey, 1982). Since normal semen contains little protective enzymes, catalase or peroxidase, the function of diluents used for preservation of boar sperm in the liquid state is aimed at providing an environment which can prevent lipid peroxide formation.

Dietary vitamin E supplementation significantly increased the number of spermatozoa per/cm³ ejaculate. The protective role of vitamin E and GSH-Px with respect to boar semen against fatty acid peroxidation had a positive influence of vitamin E supplementation on semen quality have been documented (Brezinska-Slebodzinska et al., 1995).
1.3 ROLE OF ANTIOXIDANTS

The term "antioxidant" is often implicitly restricted to compounds serving as chain breaking inhibitors of lipid peroxidation. Various "synthetic" organic antioxidants include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate and "natural" antioxidants such vitamin E, vitamin C, rosemary extracts, and flavonoids (Aruoma et al., 1993).

Normally, the body is protected against Reactive Oxygen Metabolites (ROM) and their toxic products by a wide range of known defense mechanisms. Included among the preventive systems are metal-binding macromolecules and antioxidant enzymes. Metal catalysts of ROM reactions in extracellular fluids are removed by transferrin, ceruloplasmin, and albumin. Within the body cells, GSH-Px (Se), the catalase (Fe), superoxide dismutase (SOD) enzyme which contain Mn, Cu, Zn, which remove O· and H₂O₂ by the production of H₂O as the end-product. Reduction of peroxide is accompanied by oxidation of reduced glutathione (GSH), which can be regenerated by reducing equivalents from NADPH₂ (Wilson, 1987). Despite these preventive enzymes, some O· and H₂O₂ may escape and, in the presence of Fe, may be catalyzed to more reactive ROM (Miller et al., 1993). Oxidized molecules can abstract H electrons from other molecules which subsequently produce a chain reaction. This reaction causes extensive tissue damage, which affects membrane permeability, enzyme function, and muscle tone (Miller et al., 1993).

There are several mechanisms to initiate and propagate the reactive oxygen metabolites (Miller et al., 1993).

\[
\begin{align*}
O_2 + e^- & \rightarrow O_2^- (\text{superoxide radical}) \\
O_2^- + e^- & \rightarrow O_2^{2-} (\text{peroxy anion}) \\
O_2^{2-} + H^+ & \rightarrow HO_2^- + H^+ \rightarrow H_2O_2 (\text{hydrogen peroxide})
\end{align*}
\]
Once formed, peroxides and superoxide radicals need to be removed from the cell. Without removal, these superoxide radicals and hydrogen peroxide can react with Fe^{III} to generate a more highly reactive free hydroxy radical, OH^•.

\[
\text{O}_2^- + \text{Fe}^{III} \rightarrow \text{O}_2 + \text{Fe}^{II}
\]

Peroxide can subsequently react with the ferrous iron (Fe^{II}), or possibly copper, to generate hydroxide ions (OH) and free hydroxy radicals (OH^•).

\[
\text{Fe}^{II} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{III} + \text{OH}^- + \text{OH}^•
\]

\[
\text{H}_2\text{O}_2 + e^- + \text{H}^+ \rightarrow \text{H}_2\text{O} + \text{OH}^• \text{ (free hydroxy radical)}
\]

Chain-breaking reaction occurs after the initiation of a chain reaction with the help of antioxidants (e.g. vitamin E; Murray et al., 1996).

\[
\text{ROO}^• + \text{TocOH} \rightarrow \text{ROOH} + \text{TocO}^•
\]

\[
\text{ROO}^• + \text{TocO}^• \rightarrow \text{ROOH} + \text{Non-free radical product}
\]

The body's antioxidant system includes lipid-soluble vitamin E, ubiquinone, GSH, β-carotene and water-soluble vitamin C, and urate (Sies, 1985). Although retinoic acid has been identified along with lipid-soluble antioxidants it does not appear to have major chain-breaking activity (Sies, 1985). Vitamin E terminates the peroxidative chain reaction by reacting directly with the organic peroxy radicals and being oxidized when the ROM's are quenched. Vitamin E, however, can be regenerated by vitamin C in vitro (Wilson, 1987).

In addition to regenerating vitamin E, vitamin C acts directly as a water-soluble antioxidant (Wilson, 1987).
Very high levels of glutathione (GSH) in the presence of catalytic Se compounds will act as an antioxidant and suppress chemiluminescence. This antioxidant effect of GSH can be seen in the high GSH/selenite ratio that suppresses cytolysis (Spallholz, 1994).

In 1979, several researchers began reporting on the cytotoxic effects of Se compounds on several tumor cell lines, Ehrich ascites, L1210 leukemia, and canine mammary tumor cells in mice and rats (Poirer and Milner, 1979, 1983; Milner, 1985; Kuchan and Milner, 1992). The most effective compounds in reducing tumor incidence were selenodiglutathione (GSSeSG) > selenite > selenocystine > selenate > Na₂Se > (CH3)₂Se (Poirer and Milner, 1979).
1.4 ROLE OF PROOXIDANTS

Selenium is known to constitute an active center of the enzyme GSH-Px (Rotruck et al., 1973; Flohe et al., 1973; Lawrence and Burk, 1976). Selenium also seems to have the ability to reduce the toxicity of heavy metals such as Hg and Cd by forming chemically inert complexes in the animal body (Imura and Naganuma 1978; Shamberger, 1983). On the other hand, it is well known that Se exerts strong toxicity effects (Latshaw, 1975; Underwood, 1977; Moxon and Mahan, 1981).

Although the understanding of Se metabolism has progressed rather rapidly, the mechanism of Se toxicity has not been clearly elucidated. Ganther (1968) and Hsieh and Ganther (1975) reported a sequence of reactions between selenite and sulfhydryl compounds such as glutathione (GSH) can form hydrogen selenide. Selenite cytotoxicity was enhanced by the addition of GSH or tissue extracts contributing GSH to the culture medium of cultured mammalian cells (Batist et al., 1986; Synder, 1987). Furthermore, GSH-depleted sheep erythrocytes were not lysed by selenite treatment (Young et al., 1981). Seko et al. (1989) reported the formation of active oxygen species by the in vitro reaction of hydrogen selenide, one of the reaction products between selenite and GSH, with oxygen. These results indicate that the reaction of selenite with sulfhydryl compounds may help to explain its toxic action on cells. Selenite-induced cytotoxicity in hepatocytes, as reflected by increased cellular LDH (lactate dehydrogenase) release, was preceded by a decrease in cellular GSH level and a transient increase in oxygen consumption which was subsequently accompanied by lipid peroxidation. Because selenite reacts easily with reduced GSH, the decrease in intracellular GSH content might at least partly be the result of the reaction of selenite with GSH (Kitahara et al., 1993). A selenite-induced decrease in cellular GSH has been reported in various cell lines (Batist et al., 1986; Bell et al., 1991) and in suspended rat hepatocytes (Garberg et al., 1988). Since GSH is one of the important cellular antioxidants, excess selenite may in fact induce lipid peroxidation through the depletion of cellular GSH. Prior to GSH depletion, oxygen
consumption increased. Consequently, the selenite-induced increase in oxygen consumption rate may suggest the generation of the superoxide anion by the reduction of oxygen with selenide formed in the hepatocytes. The depression of lipid peroxidation by the addition of methylmercury, which was expected to enter the cells more easily than mercuric mercury, may indicate the prevention of cell damage by inhibiting the generation of the superoxide anion through the preferential consumption of selenide by methyl-mercury to form a complex, i.e. bis (methylmercuric) selenide (Kitahara et al., 1993). Muth et al. (1967) demonstrated that Se-containing compounds (selenate, selenocystine and selenomethionine) can produce superoxides in the presence of GSH. The addition of SOD to the medium did not affect the cytotoxicity of selenite (Garberg et al., 1988). This may be explained by the inability of SOD to enter the cells. Selenite cytotoxicity has been explained in Figure 1 (Kitahara et al., 1993).

![Figure 1. Proposed mechanisms of selenite cytotoxicity (Kitahara et al., 1993).](image)

Selenite is reduced by GSH and/or GSSG reductase to generate the superoxide anion. Superoxide may enhance the Fenton type reaction by reducing ferric (Fe³⁺) to the ferrous (Fe²⁺)
iron, or by releasing ferrous iron from ferritin (Thomas et al., 1985). The superoxide-producing reactions of all Se compounds with GSH that produces superoxide are both pH and thiol concentration dependent (Spallholz, 1994). The generated active oxygen species, including the superoxide anion, \( \text{H}_2\text{O}_2 \) and hydroxyl radical, exerts toxic effects such as lipid peroxidation or a DNA strand breaks in the cells (Wu et al., 1995). DFMn (desferrioxamine-manganese complex) desferrioxamine protects cells against selenite toxicity by chelating iron and/or by scavenging superoxide anion (Kitahara et al., 1993). Cytotoxicity as revealed by cell hemolysis was as follows: selenocystine > selenite > Se dioxide. Hemolysis of rat erythrocytes by Se compounds only occurred in the presence of those Se compounds that catalytically oxidize GSH (Spallholz, 1994).

Selenomethionine and selenate were not toxic at all to rat erythrocytes at 10.0 mM (Hu and Spallholz, 1982). Ip and Ganther (1992) found that the selenoethers, selenobetaine, and dimethylselenide were less toxic and carcinostatic in rats than selenite. In addition, selenite lethal toxicity was augmented in Se and vitamin E deficient rats which were more sensitive to the chemicals generating active oxygen and to lipid peroxidation than normal rats (Dougherty and Hoekstra, 1982). Furthermore, exhalation of ethane, an indicator of lipid peroxidation in the body, was increased by selenite administration in these Se and vitamin E deficient rats.
1.5 METABOLISM AND BIOCHEMISTRY OF SELENIUM

1.5.1 Plant

1.5.1.1 Distribution

Selenium is widely distributed in minute amounts in virtually all inorganic and organic materials of the earth's crust, having an average abundance of about 0.09 ppm. Selenium is rarely found by itself in nature and has been found chiefly combined with sulfides (Cooper et al., 1970). Therefore, it is located in mineral deposits and some soil formations where a high concentration of sulfur is found. The greatest abundance of Se occurs in igneous rocks, where it occurs chiefly as selenite; in sulfides, isomorphous with sulfur; in hydrothermal deposits, commonly associated epithermally with antimony, silver, gold, and mercury; and in massive sulfide and porphyry copper deposits, where it occurs in small concentrations but in large quantities (Elkin and Merggrave, 1968). Selenium is richest in chalcopyrite, bornite, and pyrite minerals (Cooper et al., 1970). High concentrations of Se are found in sedimentary rocks such as shales, sandstones, limestones, and phosphorite rock.

Selenium is extracted from the ores commercially by treatment of anode slimes produced during the electrolytic refining of copper. The principle sources of Se are the sulfidic copper ores in Canada, the United States, and the Soviet Union (Cooper et al., 1970).

Sedimentary rocks cover more than three-quarters of the land surface of the earth and are the principal parent materials of western agricultural soils (Lakin and Davidson, 1967). It has been estimated that 58 % of all sedimentary rocks are shales, which in turn commonly contains the highest concentrations of Se (Anderson et al., 1961). The average concentration of Se in shales ash ranged from 0.24 ppm for the Paleozoic shales of Japan to 277 ppm for black shales of Permian age from Wyoming (Lakin and Davidson, 1967). Shales are the principal sources of the Se-toxic soils in Ireland, Australia, and other countries of the world (Johnson, 1975).
It has been difficult to reach a realistic estimate of the Se content of sandstones because it is often concentrated in variable organic debris in these rocks (Johnson, 1975). Lakin and Davidson (1967) obtained values ranging from 0 to 112 ppm.

The Se content of limestones is generally low, but some contain relatively high levels (Lakin and Davidson, 1967). The element has been found in seleniferous pyrite and in organic debris. The relatively high concentrations of Se in some phosphate rocks may be of importance to agriculture because of the wide use of phosphate fertilizers made from these deposits. Seleniferous sulfur is of agricultural interest as a source of Se in phosphatic fertilizers and sulfur-containing inorganic salts included in livestock diets.

Japanese and Hawaiian volcanic residue ranged from 67 to 206 ppm Se and 1,026 to 2,000 ppm Se, respectively. However, not all volcanic sulfur was found to be highly seleniferous (Lakin and Davidson, 1967).

Selenium also occurs in fossil fuels. In samples obtained in the United States, coal contained 1 to 5 ppm Se and crude oil (from Texas) 0.06 to 0.35 ppm Se (Cooper et al., 1970). A coal sample taken from a seleniferous region in China analyzed approximately 90,000 ppm Se (Levander, 1982).

Selenium is found in coal and in the resulting ash. Consequently, in connection with the construction and operation of coal-fired electric generating plants, there is concern for the Se in fly ash from coal getting into the environmental area surrounding the power plant (Wilber, 1980). Table 2 gives the Se content of various selected coals in the USA (Gluskoter et al., 1977). Consequently, Se can impact power plant emissions from the burning of coal. Selenium is emitted from the coal-fired power plants partly in the form of a vapor which can be condensed upon entry to the atmosphere. An example of Se content of coal and coal ash is as follows: coal, 0.98 ppm; bottom ash, 0.14 ppm; precipitator ash, 16.4 ppm (Wilber, 1980).

Because of the region and various types of rocks, the Se concentrations in soil is extremely variable, ranging from 0.1 μg/g to over 1,000 μg/g in seleniferous regions.
<table>
<thead>
<tr>
<th>State</th>
<th>Se, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama</td>
<td>8.1</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>6.6</td>
</tr>
<tr>
<td>Ohio</td>
<td>6.3</td>
</tr>
<tr>
<td>Illinois</td>
<td>3.2</td>
</tr>
<tr>
<td>Missouri</td>
<td>2.9</td>
</tr>
<tr>
<td>Kentucky</td>
<td>2.8</td>
</tr>
<tr>
<td>Indiana</td>
<td>2.7</td>
</tr>
<tr>
<td>Colorado</td>
<td>2.3</td>
</tr>
<tr>
<td>Wyoming</td>
<td>2.3</td>
</tr>
<tr>
<td>Arizona</td>
<td>1.2</td>
</tr>
<tr>
<td>North Dakota</td>
<td>1.1</td>
</tr>
<tr>
<td>Montana</td>
<td>.87</td>
</tr>
</tbody>
</table>

Table 2. Selenium content in whole coal in the USA

A continental-wide survey of Se in soil in the United States (912 samples) gave a range of 0.1 to 4.3 μg/g with a geometric mean of 0.3 μg/g (Shacklette et al., 1974). Berrow and Burridge (1980) demonstrated a range of Se in cultivated surface soils of 0.1 to 2 μg/g with a typical concentration of 0.5 μg/g.

Seasonal conditions can influence the amount of Se in plants, as can climate and management practices. High rainfall lowers the Se levels in the soil. When climate favors a lush forage growth, especially in areas with low soil Se levels, Se deficiencies can occur. Most of the Se is found in the leaves, due to the greater stem to leaf ratio (Jordan, 1970). Sulfur rich fertilizers can depress the uptake of Se in plants because of the downward movement of Se in the soils due to increased soil acidity (Flemming, 1962). The Se content of forages varies by species, with legumes like clovers having a lower Se content than grasses. Arthur (1971) demonstrated that soybean meal was generally lower in Se compared with rapeseed and linseed meal.
Supplemental Se allowed in the western United States (states west of and including North Dakota, South Dakota, Nebraska, Kansas, Oklahoma, and Texas) are thought to be of more concern, because these areas have, in general, lower rainfall and more alkaline soils than those of the eastern United States. They are considered to have more potential for environmental Se enrichment which could result in Se toxicosis (Edmondson, et al., 1993).

The occurrence of the vitamin E-Se deficiency problem with swine has been reported in the Midwest from several researchers (Trapp et al., 1970; Van Vleet et al., 1970, 1973). Although the severity of the problem in the field prior to its approval by FDA (1974) has been estimated to range from 3 to 10% mortality (Trapp et al., 1970), New Zealand (Hartley and Grant, 1961) other Ohio (Mahan et al., 1973b) have reported higher losses (50% to 80%) under research condition. It is well documented (Kubota et al., 1967; Patrias and Olson, 1969; Groce et al., 1972; Mahan, 1973a) that the Se content of cereal grains grown in the Midwest is below the reported requirement as established prior to 1973 (NRC).

1.5.1.2 Soil forms of selenium

Identification of the different chemical forms of Se in soils is difficult because of the small amounts of the element present (Trelease and Beath, 1949; Allaway et al., 1967) and the complexities of soil systems (Rosenfeld and Beath, 1964). The forms of Se generally present in soils are selenides, elemental Se, selenites, selenates, and organic Se compounds.

The chemical forms of Se in soils and sediments are closely related to their oxidation reduction potential, pH, and solubility (Lakin, 1961; Allaway et al., 1967; Cary et al., 1967; Allaway, 1968; Geering et al., 1968).

There is evidence that insoluble selenides associated with sulfides may occur in some soils (Trelease and Beath, 1949; Allaway et al., 1967). The low solubility of metal selenides, especially copper selenide, may lead to their persistence in agricultural soils (Allaway et al., 1967).
The principle chemical reactions of Se that occurs in soils under various weathering sediments, was summarized by Allaway (1973) and are shown in Figure 2.

SOILS

### Acid-Poorly Aerated

- **Heavy metal Selenides** $\text{Se}^{2-}$
- **Selenides** $\text{Se}^{2-}$
- **Elemental Se** $\text{Se}^{0}$

* (insoluble)

- **loss**

### Well Aerated-Alkaline

- **Selenites** $\text{SeO}_3^{2-}$
- **Selenates** $\text{SeO}_4^{2-}$

* **acid pH**
* **alkali**
* **Fe(OH)SeO_3** complexes (insoluble)
* **leaching**
* **PLANTS**

* **s** : Slow reaction
* **loss** : Process leading to loss of "biologically active" Se

Figure 2. Generalized chemistry of selenium in soils (Allaway, 1973).

Although redox potentials indicate that selenides would be oxidized to selenite in soil, the rate of oxidation is sufficiently slow to effectively stabilize this form of Se under some soil conditions (Cary et al., 1967). Elemental Se is present in small amounts in some soils and may be either an important intermediate in the oxidation of the element to a soluble form (Olson, 1967), or a transitory constituent as a result of neutral and acid soil condition during the reduction of Se under acid conditions (Allaway et al., 1967). There are indications that significant amounts of elemental Se may be oxidized by microorganisms in neutral and alkaline soils (Geering et al., 1968).

Watkinson (1962) and Allaway et al. (1967) suggested that when elemental Se is added to acid and neutral soils, it may be oxidized to selenites, which in turn react with hydrous oxides to form...
various complexes of low solubility and availability to plants. A large fraction of the Se in acid soils occurs as stable and complexes of selenites with hydrous iron oxides (Williams and Byers, 1936; Trelease and Beath, 1949; Lakin, 1961; Allaway et al., 1967). Geering et al. (1968) showed that the thermodynamically stable Se compound in acid-to-neutral soils may be a ferric selenite-ferric hydroxide adsorption complex (see Figure 2). As soil pH rises above 8, decomposition of the ferric hydroxide-selenite complexes begins, and the equilibrium solubility of selenite increases rapidly. The rate of transformation of selenite to selenate proceeds rather slowly. According to Lakin (1961), selenates are stable in an alkaline, oxidizing environment such as that found in many well-aerated, semiarid seleniferous soils. Selenates do not appear to be present in appreciable quantities in acid and neutral soils but is the form most effectively absorbed by plants. Marked increases in Se uptake by plants have resulted from application of soluble selenates to soils (Hurd-Karrer, 1935; Bisbjerg and Gissel-Nelsen, 1969; Gissel-Nielsen and Bisbjerg, 1970).

Very little is known about the nature of organic forms of Se in soils. Beath et al. (1935) suggested that soluble organic Se compounds are liberated through the decay of seleniferous plants. Williams and Byers (1936) found that soil organic matter contained water-soluble and easily recoverable organic Se compounds. The Se in seleniferous soils found by Olson and Moxon (1939) was correlated with the Se in the organic and humus fraction. Cary et al. (1967) demonstrated that organic forms of Se are more soluble under alkaline than under acidic soil conditions.

The principal factors affecting the availability of soil Se to plants have been summarized (NRC, 1971). In alkaline, well-aerated soils, Se tends to form selenates. The selenates in these soils are available to plants, and could lead to toxic concentrations in plants if the concentration of Se is high. In acid soils, a ferric iron-selenite complex is formed that is only slightly available to plants. This is the reason acid non cultivated soils rarely produce plants that contain toxic concentrations of Se. Therefore, Se is bound in acid soils as ferric selenite with a very low solubility. Soils rich in iron contribute to Se binding and reduce its availability to
vegetation. In alkaline soils, more Se is present in the soluble selenate form available to plants and the crop tissue have a higher Se content (Bennett, 1983). The broad areas of acid soils (Spodosols, Inceptisols, and Ultisols) in the eastern United States which lack seleniferous rock sources were noted to be areas with a high incidence of white muscle disease (Muth and Allaway, 1963). The amount of Se in soils is uniformly low and that the decreasing plant concentration is due to low Se availability of soils in the low-Se areas. Changes in plant concentration thus reflect the difference in Se-availability in various soils. Soils in the outer fringes of Se-adequate areas have some properties in common with neutral to alkaline soils of the Northern Plains states that produce plants with adequate Se for animals (Kubota, 1972).

Elemental Se appears to be stable in soils and, except for microbial action, is not readily oxidized to forms that are easily taken up by plants (Watkinson and Davies, 1967; Cary and Allaway, 1969). There is evidence that some of the organic Se compounds in soils are water-soluble and available to plants (Moxon et al., 1939). The uptake of soil Se by plants is dependent on plant species.

1.5.1.3 Plant absorption

There are marked differences between plant species in their ability to absorb Se from the soil. Rosenfeld and Beath (1984) have divided plants into three groups on the basis of their ability to accumulate Se when grown on high Se soils. The first two groups of plants are referred to as Se accumulator or primary indicator plants. These plants grow well on soil containing high levels of Se and thereby assist and are an asset in locating seleniferous soils. Plants in group I include many species of Astragalus, Machaeranthera, Haplopappus, and Stanleya. They normally accumulate Se at very high levels, often several thousand parts per million. Plants in group II are classified to as secondary Se absorbers. They belong to a number of genera, including Aster, Atriplex, Castelleja, Grindelia, Gutierrezia, Machaeranthera, and Mentzelia. They rarely concentrate more than a few hundred parts per million Se. Plants in group III include the cereal grains, grasses, and many weeds, that do not normally accumulate Se in excess of 50 ppm even
when grown on seleniferous soils. Pasture plants differ in their uptake of Se. Beeson and Matrone (1972) reported that white clover (Trifolium repens), buffalo grass (Hilaria belangeri), and gramma grass (Bouteloua spp.) are poor accumulators of the element. On the other hand, high sulfur-containing plants such as the Crucifera (mustard, cabbage, broccoli, cauliflower) are high Se concentrators.

There are a number of plant factors which influence the Se status in ruminants grazing forages:

1) Plant species - Se deficiencies have been associated with feeding root crops, clovers and other legumes. These findings contrast with other data which demonstrated higher levels of Se in the legume, alfalfa (Gardiner and Gorman, 1963).

2) Climate - A high incidence of myopathy was to occur in those years when above average rainfall and warmer temperatures produced lush forage growth. A higher yield with the dilution of Se and a higher stem to leaf ratio between the Se content. Because the leaf contains higher concentrations of Se, a relative deficiency was produced (Gardiner, 1962).

3) Soil fertility - fertilizers have a bearing on Se availability since sulfur ionizing phosphate fertilizer increases the downward movement of Se in the soil profile, particularly in lighter soils. Fertilizer application could therefore cause a reduction in plant Se and lead to an exhaustion of limited quantities of Se and the tying up of Se in organic matter which may not be returned to the soil in available forms (Jones and Belling, 1967).

4) Livestock management - an increased incidence of white muscle disease (WMD) has been found correlated with an increased stocking rate (Gardiner, 1962). As crop productivity increased stocking rate could hasten Se depletion from low Se soils by tying up larger amounts of the element in plant and animal products, primarily feces.
5) Removal of Se from the food chain - large quantities of grain are consumed by humans and exported which results in a permanent loss of Se from the land. Conceivably, such losses could lower plant levels to the deficiency range in marginal areas.

The tolerance or toxicity to soil Se is quite variable (Brown and Shirft, 1982). Some plants grow only in soils of high Se content and accumulate concentrations up to a few thousand ppm.

1.5.1.4 Formation of plant selenium

In early studies on the alkali disease syndrome it was found that high levels of Se were associated with the protein component of grains (Franke and Painter, 1936; Horn et al., 1936). Selenium-accumulating plants, like some Astragalus species, require Se for their growth. These plant are also able to absorb some of the more unavailable forms of Se (Lannek and Lindberg, 1975). The wide variation in the Se concentration of cereals and grains is due to differences in the amount of soil Se available for uptake by plant (phytoavailability). Yang et al. (1983) reported that the Se content of corn collected in the People's Republic of China ranged from .005 to 8.1 ppm Se, depending upon whether the samples came from areas with soils that were poor or rich in phytoavailable Se.

In nonaccumulator plants, such as wheat, most of the Se is incorporated into the protein component, selenomethionine being the predominating compound. This is different from accumulator plants where Se is not ordinarily found in the protein fraction. Selenocystathionine and Se-methylselenocysteine are the major Se compounds in accumulator plants (Shirft, 1969). Distribution of Se in corn grain in various areas of the United States (Ullrey, 1974) is presented in Table 3.
<table>
<thead>
<tr>
<th>Origin</th>
<th>No. of Samples</th>
<th>Mean (ppm)</th>
<th>Range (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illinois</td>
<td>31</td>
<td>.05</td>
<td>.02 - .15</td>
</tr>
<tr>
<td>Indiana</td>
<td>17</td>
<td>.04</td>
<td>.01 - .15</td>
</tr>
<tr>
<td>Iowa</td>
<td>26</td>
<td>.06</td>
<td>.02 - .32</td>
</tr>
<tr>
<td>Michigan</td>
<td>42</td>
<td>.02</td>
<td>.01 - .09</td>
</tr>
<tr>
<td>Minnesota</td>
<td>23</td>
<td>.09</td>
<td>.02 - .19</td>
</tr>
<tr>
<td>Missouri</td>
<td>4</td>
<td>.05</td>
<td>.02 - .09</td>
</tr>
<tr>
<td>Nebraska</td>
<td>6</td>
<td>.35</td>
<td>.04 - .81</td>
</tr>
<tr>
<td>North Dakota</td>
<td>5</td>
<td>.19</td>
<td>.09 - .26</td>
</tr>
<tr>
<td>Ohio</td>
<td>5</td>
<td>.09</td>
<td>.06 - .15</td>
</tr>
<tr>
<td>South Dakota</td>
<td>9</td>
<td>.40</td>
<td>.11 - 2.03</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>5</td>
<td>.04</td>
<td>.02 - .13</td>
</tr>
</tbody>
</table>

Table 3. Selenium concentration in corn in the USA (Ullrey, 1974)
1.5.2 Animal

1.5.2.1 Absorption

The delivery of dietary Se from the digestive system subsequently to body tissue varies greatly between the ruminant and the non-ruminant.

In swine, no absorption occurs in the stomach or in the first part of the small intestine. The greatest absorption has been shown to occur in the last part of the small intestine, the cecum, and the colon (NRC, 1983). In the nonruminant, most of the dietary selenite remains in the oxidized form as it enters the small or large intestine. The absorption of selenite or selenomethionine in rats occurs mainly from the duodenum, with slightly less being absorbed from the jejunum and ileum, and practically none from the stomach (Whanger et al., 1976).

In ruminants, the rumen reduces some of the inorganic selenite to selenide by the hydrogenation process that occurs in the rumen (Mahan, 1995). The resulting selenide is not absorbed in the rumen nor later in the intestinal tract.

The mechanism of Se absorption in the G.I tract and its transportation in the blood stream is largely unknown. Absorbed Se is transported largely associated with GSH-Px in erythrocytes with plasma proteins playing an important role (Sandholm, 1975). Even though there are differences in the amount of Se associated with GSH-Px in erythrocytes between primates and other animals, the metabolism of selenite by blood in vitro is similar.

Another hypothesis is that selenoprotein (Se-P) transports Se from the liver to other tissues (Burk and Hill, 1993). In the rat, Se-P contains 65% of the plasma Se (Read et al., 1990). The existence of a plasma selenoprotein other than eGSH-Px has been known for over 15 years, but the purification and characterization of (Se-P) has been accomplished recently. According to cDNA for Se-P (Hill et al., 1991), there are ten TGAs (UGAs in mRNA) in the open reading frame (ORF), indicating that the protein contains ten selenocysteine residues in its primary structure. It is the only selenoproteins characterized so far that contains more than one selenocysteine per
polypeptide chain. However, Se-P mRNA has been detected in several tissues, thus indicating that it is synthesized in sites other than the liver (Hill et al., 1992). Also the half-life of its Se is not affected by the Se status of the animal (Burk et al., 1991). Consequently, these observations in Se-P make a transport role of Se unlikely (Burk and Hill, 1993).

The uptake and release of selenite by bovine (Jenkins and Hidiroglou, 1972a) or human erythrocytes (Lee and Yang, 1969) involve sulfhydryl group, and the binding of selenite to plasma proteins depends upon the presence of erythrocytes (Lee and Yang, 1969; Sandholm, 1974; 1975). Selenite has been shown to react non-enzymatically at pH 7.4 with reduced GSH to form selenodiglutathione (GSSeSG) as the most stable product. Consequently, the uptake and subsequent metabolism of selenite was dependent upon GSH (Gasiewicz and Smith, 1978).

$$\text{H}_2\text{SeO}_3 + 4\text{GSH} \rightarrow \text{GSSeSG} + \text{GSSG} + 3\text{H}_2\text{O}$$

In cell-free systems, GSSeSG has been found to be further reduced by NADPH and yeast glutathione reductase to form the selenopersulfide (GSSeH).

$$\text{GSSeSG} + \text{NADPH} + H^+ \xrightarrow{\text{glutathione reductase}} \text{GSSeH} + \text{GSH} + \text{NADP}^+$$

The labile selenopersulfide can be further reduced by NADPH and glutathione reductase to form an acid-volatile Se moiety, presumed to be H$_2$Se.

$$\text{GSSeH} + \text{NADPH} + H^+ \xrightarrow{\text{glutathione reductase}} \text{H}_2\text{Se} + \text{GSH} + \text{NADP}^+$$

Hydrogen selenide is unstable and may undergo oxidation to elemental Se, or combined with tissue macromolecules such as albumin, or be metabolized to other Se compounds. The pathway of selenite metabolism in animals leading to the formation of methylated selenides is believed to be via the H$_2$Se intermediate.
However, selenomethionine can be incorporated directly into body proteins in place of methionine because met-tRNA can not distinguish between methionine and selenomethionine. Alternatively selenomethionine can be converted through the transsulfuration mechanism to selenocysteine, which in turn is degraded to hydrogen selenide (H$_2$Se) by the enzyme $\beta$-lyase (Ip, 1998).

Hydrogen selenide (H$_2$Se) is the precursor for supplying Se in the active form for the synthesis of selenoproteins. The further metabolism of H$_2$Se involves sequential methylation by S-adenosylmethionine to methylenol, dimethylselenide and trimethylselenonium ion (Ganther, 1986).

Selenium metabolic pathway is presented in Figure 3.

![Selenium metabolic pathway](image)

Figure 3. Selenium metabolic pathway (Ganther, 1986)
After the erythrocytes have metabolized the Se from selenite, specific carrier proteins seem to take up the Se and conveying throughout the body (Sandholm, 1974). Labeled Se has been shown to become loosely bound to albumin and then to the globulin fractions (Sandholm, 1973). In mice plasma, the protein-bound Se is mainly located in the albumin, with smaller amounts situated in the α- and β-globulins (Sandholm, 1974).

In human plasma, the lipoproteins appear to be different Se binding proteins. The important Se binding proteins seemed to be the β-lipoprotein and an unidentified protein (lying between the α1- and α2-globulin) regions (Sandholm, 1975). This is consistent with the findings of Burk (1974), who showed that up to 16% of plasma $^{75}$Se was found in Very Low Density Lipoproteins (VLDL) after the administration of $^{75}$Se-selenite to patients. Lee and Yang (1969) reported that uptake of $^{75}$Se$_3$ by bovine, chick, and ovine erythrocytes and subsequent release of $^{75}$Se activity was much slower than human red blood cells. Porter et al. (1979) reported that the $^{75}$Se bound to human plasma proteins is absorbed by lymphocytes in preference to selenite, suggesting that plasma proteins function as carrier of Se to these lymphocytes. Selenium bound to proteins is absorbed by lymphocytes more readily (3 to 4 times) than is selenite (Porter et al., 1979). Jenkins and Hidiroglou (1972a) also demonstrated that most of the $^{75}$Se$_3$ associated with the plasma proteins was loosely bound before the ion has an opportunity to enter the red blood cells. Incubation of a higher concentration of selenite with either whole blood or cells markedly enhances erythrocyte uptake $^{75}$Se (Jenkins and Hidiroglou, 1972a).

When Se was transported by various carriers, the cells, mainly in the liver, take up Se (max. concentrations at about 15 minutes), after which the unused portion is probably expelled in to the plasma and excreted as methylselenides (Sandholm, 1973).

Transport and metabolism of selenate and selenomethionine in erythrocytes has received less attention than selenite. When high level (15 μg/100 ul) of $^{75}$Se-selenite, $^{75}$Se-selenate, and $^{75}$Se-selenomethionine were added to whole blood, $^{75}$Se activity in selenite treatment occurred in RBC and plasma protein approximately 50% and 40%, respectively. Most of the $^{75}$Se activity of selenate and selenomethionine, however, occurred in plasma (< 80%), plasma protein
(< 15%), and RBC (< 5%) (Jenkins and Hidioglou, 1972a). This represents that the mechanism of the absorption and transport both in selenate and selenomethionine may be different from selenite. Small amounts of selenate and selenomethionine were rapidly taken up by cells and maintained at a constantly low level (Jenkins and Hidioglou, 1972a). It is likely that bidirectional transport of these forms of Se is by simple diffusion has been reported for Cl⁻, HCO₃⁻, SO₄²⁻, and monobasic amino acids (Prankerd, 1961).

The absorption of Se is lower in ruminants than in nonruminant animals. The absorption of Se as sodium selenite was found to be 77 % in swine as compared to only 29 % for sheep (Wright and Bell, 1966). Wright and Bell (1966) demonstrated in sheep that the duodenum is the main site of absorption with ⁷⁵Se at physiological levels and no absorption from the rumen or abomasum.

By use of the everted intestinal sac technique, McConnell and Cho (1965) found that selenomethionine was transported against a concentration gradient, whereas selenite and selencystine were not. The transport of selenomethionine was inhibited by methionine (McConnell and Cho, 1965), but the transport of selenite and selenocystine was not inhibited by their respective sulfur analogues. It was suggested that Se absorption was enhanced by feeding a high-protein diet, but the different forms of Se provided in the high- and low-protein groups complicated its interpretation (Levander, 1986).

Organic Se compounds, such as selenomethionine, are actively transported through the enterocyte of the small intestine (McConnell and Cho, 1965) and may be incorporated directly into tissue and accumulate to high levels (Scott, 1973). However, the inorganic forms, such as selenite or selenate, are absorbed passively by nonruminant (McConnell and Cho, 1965). Consequently, in the nonruminant the inorganic Se form is better absorbed than the organic form (Mahan, 1995). It is estimated that 80% of the dietary inorganic Se is absorbed (Stewart et al., 1978). Retention of inorganic selenite may be limited by available tissue Se binding sites, although some biotransformation to organic forms occurs, which are then incorporated directly into tissue proteins (Glover et al., 1979). Although absorption of selenites, selenates and
organic Se compounds appear to be efficient, metal selenides and elemental Se are poorly absorbed.

Soluble Se compounds are efficiently absorbed from the gastrointestinal tract, since rats absorbed 92, 91, and 81% of tracer doses of selenite, selenomethionine, and selenocystine, respectively (Thomson and Stewart, 1973; Thomson et al., 1975).

There appears to be no homeostatic control mechanism for Se absorption of selenite by rats, since at least 95% was absorbed by rats over a range of dietary Se intakes from deficient to mildly toxic levels (Brown et al., 1972). Numerous balance studies conducted with humans have shown that the apparent absorption of dietary Se ranges between 55 and 70% (Levander, 1986).

Levander et al. (1981) reported that a sudden decrease in Se intake can reduce its apparent absorption, presumably because of a continued and relatively high rate of endogenous losses, principally through the bile (Levander and Bauman, 1966).

![Diagram of Selenium Utilization](image)

**Figure 4. Utilization of absorbed selenium (Mahan, 1995).**

* Dotted lines refer to where inadequacies will affect formation of the seleno-protein.
Most dietary intake of Se occurs via plant foods but there is some Se consumed from fish and seafood products. There is considerable variability in the Se levels in plants due to various soil conditions, whereas levels are more consistent in animal tissues. The general metabolism of Se in the body is depicted in Figure 4.

The effect of food processing on the different chemical forms of Se have not been established. There is, however, a considerable loss of volatile Se compounds during cooking (Ganapathy et al., 1978). It has been noted that the availability of Se following ingestion is higher originating from plant origin than from animal origin (Cantor et al., 1975a). Selenium in fish, for example, can be chelated with heavy metals which reduces its availability (Mahan and Moxon, 1978).

1.5.2.2 Body retention and tissue distribution

Absorbed Se is widely distributed to the tissues via the blood. The highest concentrations occur in the liver and kidneys. Jenkins and Winter (1973) demonstrated that kidney contained the highest Se concentration of followed by the liver and other glandular organs with lower levels in the cardiac and skeletal muscle. Jenkins and Hidioglou (1973) suggested that the organ Se stores may not be readily available to animals.

New Zealand workers (Cousins and Cairney, 1961; Andrews et al., 1968) found that the greatest accumulator of Se is the kidney cortex. However, other researchers (Glenn et al., 1964; Gardiner, 1966; Gabbedy and Dickson et al., 1969) reported liver Se levels of 2 to 3 fold compared with those of the kidney under long term toxicity studies. It should be noted that Cousins and Cairney (1961), Andrews et al. (1968) reported the highest levels of Se in the kidney cortex. Hoffman et al. (1973) demonstrated that the Se concentration in kidney cortex in sheep had a uniform Se content. When dietary Se intake was low, the kidney of lambs had higher Se concentration than the liver. When the dietary intake of Se was increased, the liver usually increased its concentration of Se than more so the kidney (Oh et al., 1976).

Concentration of Se in tissue, particularly the liver, has been used in establishing Se status of the animal (Ammerson and Miller, 1975).
Medinsky et al. (1985) demonstrated that the distribution of Se to various organs was similar when rats were fed different levels of Se (1.3 v.s. 4.6 mg) from either selenious acid or sodium selenite. However, the rate of clearance from the tissue was slower when low level of Se (0.02 mg/m^3) was provided to rats.

The distribution and retention of Se between the liver, blood, and other tissues are generally similar for each chemical form (from selenious acid or sodium selenite) but in the lung, Se was retained for a longer time than was selenious acid (Medinsky et al., 1985). Differences in the clearance rates of Se were found in studies of the whole-body retention of radiolabeled Se injected into rats along with varying amounts of stable carrier Se (Burk et al., 1972). As the amount of carrier administered increased, the half-time for Se retention decreased. This is a physiologically significant mechanism in that low doses Se are conserved by biological systems and at higher doses it is excreted at a faster rate, which serves to minimize the potential for toxicity (Medinsky et al., 1985).

Not only is the tissue content of Se dependent upon its level in the diet, but also upon the chemical form provided. In general, Se is deposited in all tissues at higher concentrations when present in diets as organic rather than as inorganic Se. Ku et al. (1972b) fed two swine rations, organic and inorganic Se form, both containing 0.44 ppm, but approximately 4 fold as much Se was deposited in the longissimus muscle when pigs were fed the diet with natural Se diet than those receiving selenite. When natural Se was provided, muscle samples were higher in Se in swine (Lindberg, 1968) and chicks (Scott and Cantor, 1971) compared with inorganic Se supplementation. Inorganic and organic Se from brewer's grains or distillers' grains with solubles results in similar weekly serum response curves within each Se level with 4-week-old weanling swine, while Se contributed by fish meal had lower levels (Mahan and Moxon, 1978). At a .4 ppm dietary Se intake, muscular tissue Se levels were higher, compared to when the selenite source was fed. When fish meal or brewers' grains were fed, the nonmuscular tissue (liver, kidney, and testes) had similar Se concentrations for all groups. Fish meal provided the poorest Se retention of all test products evaluated. Mahan and Moxon (1978) demonstrated that
fish meal which contained heavy metal (Hg) was complexed with Se. Thus the reason for the
lows Se retention when fish meal was added to the diet.

When .1 ppm of sodium selenite was provided to grower-finishing pigs, a 60-day withdrawal of
Se supplementation had no effect on the Se concentration in longissimus muscle (Ku et al.,
1972b). This demonstrated that a Se supplement withdrawal period before marketing is not
necessary.

In poultry, Se is also distributed widely throughout the body tissue, with the highest
concentrations found in the liver and kidney, followed by muscle, bone, and plasma (Levander,
1986). Within 2 months after Se supplementation was withdrawn, egg Se levels declined by 50
% (Kinder et al., 1995). Latshaw (1975) reported that Se (selenite form) was deposited more
readily in the yolk than in the albumen. This observation is most likely due to the timing and
location of egg protein production. The synthesis of egg white protein occurs in the magnum
(Anfinsen and Steinberg, 1951), whereas egg yolk proteins are synthesized in the liver
(Greengard et al., 1965). Thus, changes in dietary Se would have an effect in the egg albumen
before affecting the yolk Se content (Kinder et al., 1995). Selenium given as selenomethionine
resulted in higher content in broiler muscle than selenite or selenocystine (Osman and Latshaw,
1976). Latshaw and Biggert (1981) also showed that dietary selenomethionine resulted in higher
egg Se concentrations than dietary selenite. Selenium given as selenomethionine or as
seleniferous wheat resulted in higher Se content than when Se was given as selenocystine,
Astragalus Se, or fish meal (Latshaw and Osman, 1975). When Se was supplied as a natural
feed ingredient (.42 ppm) for hens, it causes higher muscle, liver, and egg Se content than when
the same amount was supplied predominantly as selenite (Latshaw, 1975), results consistent
with tissue data from sheep, cattle, and swine.

In human tissues, higher Se concentrations were found in the lungs with about equal
concentrations in kidneys, spleen, pancreas, muscle and brain (Yukawa et al., 1980). Whole
body Se content was approximately 3 to 6 mg in the low Se region of New Zealand (Stewart et
al., 1978).
Hunter et al. (1990) reported that toenail Se can be a good indicator of Se status in human because they are less prone to superficial contamination than hair. Toenail Se content declined with age and among cigarette smokers but was not affected by alcohol consumption.

Viegas Crespo et al. (1995) reported that polyunsaturated fatty acids (PUFA) increased when .5 ppm Se was fed to rats. Ursini et al. (1985) demonstrated that the phospho-lipid hydroperoxide glutathione peroxidase interfered more directly with the protection of membrane PUFA.

Selenium retained in the respiratory tract can be absorbed into the blood and subsequently transported by mucociliary clearance to the gastrointestinal tract, whereupon it is either absorbed or excreted in the feces. Absorbed Se is bound with plasma proteins in the blood (Lee and Yang, 1969; Jenkins and Hidiroglou, 1972a; Burk, 1974; Sandholme, 1973, 1974, 1975; Gasiewicz and Smith, 1978; Porter et al., 1979) and either excreted in the urine or transported to the liver for further metabolism (Medinsky et al., 1985).

Goehring et al. (1984b) reported that hair Se concentration in pigs increased quadratically, while blood Se increased linearly as the level of supplemental Se increased. Wahlstrom et al. (1984) reported that the Se content of hair in swine was related to the dietary Se level. Hair color seemed have an important relationship to its concentration. Black and white haired pigs contained more Se than from red pigs. Olson et al. (1954) reported that pigs fed 20 ppm Se had less than 10 ppm Se in the hair. Although these pigs were fed the highest dietary Se level in this experiment, total Se intake was less than for pigs that consumed more of the diets containing lower levels of Se (Olson et al., 1954). Herigstad (1972) and Harrison et al. (1983) reported no abnormality in cell volume or hemoglobin level of pigs suffering from Se poisoning. There were no difference between barrows and gilts in hair Se concentration and blood composition (Goehring et al., 1984). Willett (1987) demonstrated that Se in blood and urine was substantially more responsive to short-term changes in Se intake, but they were a less accurate for of long-term Se exposure.
Hidirogloiu et al. (1968) demonstrated that cows with hair Se concentrations between .06 and .23 ppm produced calves with white muscle disease, while no white muscle disease was found in calves from cows with hair Se concentration greater than .25 ppm. Continuous intake of 5 ppm Se by cattle may result in selenosis. Hair Se concentrations of 5 to 10 ppm of cattle may indicate Se toxicity. Cell volume and hemoglobin levels were not affected by dietary Se level (Goehring et al., 1984b).

1.5.2.3 Excretion

The primary routes of Se excretion are in urine, feces, and lung exhalation. The amount and distribution of Se eliminated depends on level of Se intake, the form of Se provided, and dietary Se antagonists. Exhalation seems to be a major route of Se excretion only when toxic concentrations are consumed (NRC, 1983). Urine is normally the major pathway of excretion in nonruminant animals and humans when inorganic Se is provided. The amount of Se excreted in the urine is closely related to dietary intake, with rat studies suggesting that the shutting down of urinary Se excretion below the dietary threshold is an important mechanism for conservation during periods of low Se intake (Burk et al., 1973). Janghobani et al. (1990) reported that urinary Se excretion increased rapidly and plateaued within a few days after rats were fed high levels of selenite. The increase in urinary Se during selenite supplementation was associated with a dramatic rise in urinary trimethylselenonium ion (TMSe) content. This metabolite represented only 2% of the urine Se in control rats but 35 to 40% in Se supplemented animals. Morris and Levander (1988) showed that when weanling rats were fed a nonpurified diet and given water containing 5 or 10 μg Se (as selenate)/ml for 8 wk, TMSe accounted for 7 or 25% of urinary Se, respectively.

Hitchcock et al. (1978) demonstrated that more Se was lost in the feces of weanling pigs when the Se source was derived from grain. Mahan and Parrett (1996) reported that fecal Se excretion was higher than urinary Se when Se-enriched yeast (selenomethionine) was provided.
Janghorbani et al. (1990) demonstrated that total body Se increased only slightly after selenite supplementation. This increment was small compared with the observed difference between Se intake and combined losses in urine and feces. This suggests the possible existence of additional route(s) of Se elimination.

Part of the Se ingested by animals will be excreted in the feces in an insoluble form, possibly elemental Se or heavy metal selenide complexes (Cousins and Caimey, 1961). Unaccounted Se thus represented about 35% of the intake. Janghorbani et al. (1990) demonstrated that pathways other than urinary and fecal excretion might account for a substantial portion of Se loss. Selenium may be lost through skin and hair, but the importance of these losses is uncertain. Another potential excretory pathway is via the respiratory tract as dimethylselenide (DMSe) or other volatile metabolites (Hirooka and Galambos, 1966; Ganther, 1986). Hirooka and Galambos (1966) showed that following the intravenous administration of selenate up to 250 g/rat or at a dose age of 80 μg/kg body weight only 0.6 ± 0.2% appeared in the breath in 24 h. However, when the dose was increased to 1,400 μg Se from selenate or selenite/kg body weight, breath Se excretion was 17.8% ± 1.5 and 22.3% ± 1.2, respectively. Therefore, excretion of Se via the lungs appeared to occur particularly when higher levels of Se are fed. Diplock (1976) reported that at low Se levels, the Se is eliminated primarily in the urine, with only small amounts excreted in the bile and through the lungs. At high doses, more of the Se is eliminated via the lungs as volatile dimethylselenide.

Exhalation of the volatile Se metabolite, dimethylselenide, is indication of acute toxicity. The percentage exhaled is proportional to the amount of Se administered (Medinsky et al., 1981). The exhaled volatile compound, dimethyl selenide, is an intermediate product of Se metabolism, which is exhaled only when its formation exceeds the rate of further methylation to TMSSe, a urinary Se metabolite (Glover et al., 1979).

Transfer of absorbed Se into the gastrointestinal tract can be either by gastric or intestinal secretory fluid or by biliary excretion (Levander and Bauman, 1966). In general, bile is considered to be only a minor route of excretion of Se (Levander and Bauman, 1966).
Selenium excretion in ruminants is dependent on the method of administration. When Se is provided orally to ruminants, most is excreted with the feces, whereas more of the injected Se is excreted in the urine (Wright and Bell, 1966). In ruminants, microbial reduction of Se compounds to insoluble forms occurs following ingestion, reducing the absorption resulting in higher fecal losses. Therefore, most Se in the feces has not been absorbed from the diet, plus a small amount from the biliary, pancreatic, and intestinal secretions (McDowell, 1992).

When manure is applied to the soil, it will be subject to the chemical, biological, and geologic forces that convert one form of Se to another. Some Se may be volatilized from decaying organic Se from plants and microbes in the soil, some will enter the terrestrial food chain, some will be retained by crops and subsequently removed by harvest, whereas some will be present in runoff or absorbed to clays and organic matter. Few studies on the environmental effects of Se supplements given to livestock have been performed, largely because the total amount of Se involved is small, compared with that from other sources (Edmondson et. al., 1993).

1.5.2.4 Deficiency

Selenium deficiency is related to several nutritional disease condition in animal and humans. The pathological changes found in animals include growth retardation, skin lesions and hair loss, visual defects, reproductive disorders, pancreas atrophy, liver necrosis and dystrophy of the skeletal muscle and of the heart muscle. The occurrence, in animals of Se-responsive endemic deficiency diseases in various parts of the world is an excellent example of the interrelation between the geochemical environment and geographic pathology of a nutritional inadequacy. White muscle disease in sheep and cattle is a widespread, naturally occurring form of muscular dystrophy caused by a Se deficiency (Schwarz, 1976). In humans, a low-Se status may lead to cardiomyopathy and muscular disorders. A role of Se in cardiovascular disease, osteoarthropathy and cancer has been discussed, but a direct relationship between Se deficiency and these diseases have yet to be established (Behne et al., 1994).
Selenium deficiency markedly affects glutathione metabolism and some glutathione-dependent enzymes. Selenium deficiency results in drastic decline in Se-dependent GSH-Px activity, which can produce a rise in cellular H$_2$O$_2$ concentration. Higher levels of H$_2$O$_2$ can still be disposed of through cellular catalase activity (Jones et al., 1981). The higher steady-state level of H$_2$O$_2$ probably will emerge as an indication of a Se deficiency. This has not been measured directly. It is not clear what effect the loss of Se-dependent GSH-Px has on fatty acid hydroperoxides in the cell because of the uncertainty over their metabolism and the presence of the nonSe-dependent GSH-Px. Selenium-dependent GSH-Px may play a metabolic regulatory role as proposed by Sies and Moss (1978). Consequently, a Se deficiency may decrease the ability of the mitochondria to adjust to changes in substrate concentrations and result in a higher H$_2$O$_2$ production.

Selenium deficiency can also cause an increase in hepatic glutathione S-transferase activity (Burk, 1983). This should provide more binding sites to allow increased "storage" of compounds such as bilirubin, heme, and other organic anions. More importantly, the increased glutathione synthesis should increase the ability of the liver to detoxify substances via the glutathione conjugation pathway. The Se deficiency-induced increase in hepatic glutathione synthesis depletes cellular cysteine, so it may impair cellular process such as protein synthesis that require cysteine. It increases plasma glutathione concentrations due to an increased rate of glutathione release by the liver (Burk, 1983).

Van Vleet et al. (1973) demonstrated an effective preventative injection dosage for Se deficiency of .06 mg Se per kg body weight in 1-week-old piglets. Mahan et al. (1973b) reported excellent results when 1 mg of Se was intramuscularly injected in 3-to 4-week-old swine.

Deficiencies of Se in cattle and sheep have been observed under natural grazing conditions in many countries of the world. Overt signs of Se inadequacy such as white muscle disease (nutritional muscular dystrophy) occur primarily in young calves or lambs when born to Se deficient dams. Infertility has increased in ewes grazing pastures low in Se. Selenium deficiency have not generally occurred in older animals such as finishing beef cattle and
lactating beef cows (Ammerman and Miller, 1975). Subclinical deficiencies of Se are not easily
determined and an inadequacy of the element may be limiting maximum animal performance
under certain circumstances of drylot feeding.

Selenium was originally considered as a toxic element, but in 1957 Schwarz and Foltz
recognized Se to be the effective component of “factor 3” which prevented liver necrosis in rats.
Schwarz and Foltz (1957) further demonstrated that Se prevented exudative diathesis in chicks.
Selenium deficiency in chicks caused reduced egg production and hatchability in poultry (Cantor
and Scott, 1974) poor growth, and increased mortality and gizzard myopathy in young turkey
poults (Scott et al., 1967). Pancretic fibrosis also occurred in severe Se deficient chicks
(Noguchi et al., 1973; Thomson and Scott, 1970). Chicks fed the Se-free diet showed severe
degeneration and fibrosis of the pancreas even when the diet was supplemented with all
nutrients known to be required, including high levels of vitamin E (Thomson and Scott, 1970).
Bartholomew et al. (1998) demonstrated that heterophils and monocytes were increased in Se
deficient chicks, whereas, lymphocytes, basophils, and Hb decreased. Selenium deficient
chicks had coagulative necrosis of myocytes accompanied by scattered hemorrhage. In
addition, the ratio of myeloid-to-erythroid (M:E) from erythroid hyperplasia in femoral bone
marrow decreased in Se deficient chicks because of increased immature erythroid cellular
elements (Bartholomew et al., 1998).
1.6 TOXICITY OF SELENIUM

Selenium was initially recognized as a toxic element long before its role as an essential nutrient was discovered. Rosenfeld and Beath (1964) provided quotes from the writing of Marco Polo suggesting that he encountered problems in "beasts of burden" during his travels through western China about 1295. The same authors refer to a report written in 1857 by T. C. Madison, an army surgeon, in which Madison describes conditions at Fort Randall, South Dakota that resulted in the sloughing of hooves, mane, and tail in cavalry horses. This condition or disease, was termed "alkali disease" or "blind staggers" and resulted in high livestock losses in a number of western states. It was not until 1934 that Se was identified as the causative toxic agent (Franke, 1934).

Selenium toxicity has been assumed to be at least partially due to the replacement of chemically similar sulfur (S) atoms in biomolecules with Se, thus altering the functions of these biomolecules (Axley and Stadtman, 1989). For example, when levels of selenomethionine are elevated in response to high Se concentrations, selenomethionine is incorporated into nascent proteins in place of methionine, a change that could alter the structure and reactivity of these proteins (Axley and Stadtman, 1989). These effects would be additive, and the greater the percentage of selenomethionine residues in a protein the greater the total effect. Incorporation of one or a few selenomethionine residues in polypeptide molecule may be safely accommodated, whereas more may precipitate the selenosis condition (Axley and Stadtman, 1989). Selenium toxicity in livestock is invoked by ingestion of both primary and secondary Se accumulator plants. Experimental chronic Se toxicity in animals is known to affect the major organs including the liver, spleen, kidneys, heart, and pancreas (Spallholz, 1994). Multiple factors contribute to chronic experimental Se toxicity including animal species, dietary Se form, quality of dietary protein, dietary acclimation, and dietary Se concentration (Spallholz, 1994).

Selenium toxicosis (selenosis) is found in either acute and chronic form. Ingestion of Se-accumulating plants (including Astragalus spp and Stanleya spp, which can contain 1,000 to
3,000 mg of Se/kg of dry weight. An accidental oversupplementation with high Se level from these sources can cause acute toxicosis with respiratory distress, ataxia, diarrhea, and death (blind staggers). The acute toxicity of Se compounds primarily affect the central nervous system similar to that of strychnine poisoning. Animals die rapidly, often within a few minutes after the injection in very acute tonic, tetany-like, generalized cramps, apparently because of postsynaptic enzymatic interference. Harrison et al. (1983) reported paralysis in animals due to focal symmetrical poliomalacia. The specificity of the site of the malacia is of interest, since in both sheep and swine the lesions appear to be confined to the ventral horns of the cervical and lumbar spinal intumescences. Aside from deaths because of acute central nervous system toxicity, a number of animals will die within 1 to 3 days from parenchymal damage to other organs. The acute intraperitoneal (or subcutaneous) toxicity of selenite-Se is such that 4.1 mg per kg of body weight will kill 50% of the animals (LD$_{50}$ = 4.1 mg). In acute poisoning, blindness, abdominal pain, salivation, grating of teeth, some degree of paralysis, respiratory failure and starvation due to loss of appetite are symptoms found in cattle, horses and pigs (Ekermans and Schneider, 1982).

Chronic toxicoses results from the long-term consumption of herbage containing 5 to 20 mg Se/kg (NRC, 1984). Chronic Se poisoning is recognized by animal dullness, lack of vitality, roughness of coat, hair loss (alopecia) from the mane and tail of horses and body of pigs, soreness and sloughing of hooves, leg stiffness and lameness due to erosion of the joints of long bones, body emaciation (alkali disease), atrophy of the heart, cirrhosis of the liver and anemia (Ekermans and Schneider, 1982). The distinctive histological changes that develop hooves, particularly in stratum medium, may account for the dystrophic digital lesions in selenosis. These lesions were accompanied by mild to marked hyperplasia and parakeratosis in laminar epithelium and, to a lesser extent, in the coronary epidermis and loss of normal abrupt coreum (O'Toole and Raisbeck, 1995). Chronic selenosis caused the reduction of the size of the inner root sheath which suggests that selenosis may induce defective terminal maturation of keratinocytes in the hoof. Epithelium from hair follicles and hooves is similar, particularly in the
relative proportions of low-sulfur keratin polypeptides, high- and ultrahigh-sulfur proteins, and high-glycine/tyrosine proteins. High-sulfur proteins of hair, which are poorly characterized because of methodological difficulties, probably form the amorphous interfibrillar matrix that separates ordered filaments of keratin, including the 5 major hard keratin polypeptides of human hair and nail (O'Guin et al., 1989). Given the chemical similarities of Se and S, it seems reasonable that the epithelial lesions of alkali disease ultimately results from the substitution of Se for S in the cysteine residues of high-sulfur proteins, with concomitant weakening of disulfide cross-linking (Yager and Scott, 1993).

Selenomethionine results in higher concentrations of Se in most tissues than when sodium selenite and other inorganic forms of Se are fed. The steer with the most severe digital lesions also had higher concentrations of Se in its hair, liver, and kidney. These results indicate that for a given dose of Se, selenomethionine is more likely to cause alkali disease than sodium selenite (O'Toole and Raisbeck, 1995).

Rats tolerated higher dietary levels of inorganic Se in wheat-based diets than in corn-based diets (Palmer et al., 1983).

Selenium toxicity occurred at identical levels when animals were fed inorganic Se as selenite or selenate. Chronic ingestion of either selenite or selenate in the rat, at the same Se dietary level, exhibits nearly equivalent toxicity (Wilber, 1980; Brasher and Ogle, 1993). Selenate, a noncatalytic and almost noncytotoxic Se compound in vitro, has about the same in vivo toxicity as selenite when fed to rats. This is because selenate in vivo is reduced to selenite, which then enters the mainstream of Se metabolism, accounting for its nearly equal toxicity symptoms (Young et al., 1982).

Dietary ingestion of organic Se compounds, however, can exhibit wide differences in its toxicity. In comparison to selenite, on an equivalent Se basis, selenocystine toxicity is approximately equal to selenite (Martin and Hurlbut, 1976). Selenomethionine in rats is less toxic than selenite with the L-isomer being only slightly more toxic than the D-isomer (McAdam
and Levander, 1987). Other selenoethers, dimethylselenide, trimethylselenonium ion, and selenobetaaine are not very toxic in comparison to selenite (McConnell and Portman, 1952).

The hypothesis of Se toxicity based upon in vitro studies (Spallholz and Whittam, 1992; Spallholz et al., 1993) was that:

1. Selenium compounds, i.e., selenite and Se dioxide, react with GSH and other thiols to form selenotrisulfides that will ultimately react to form selenotrisulfides. This product will ultimately react to produce superoxide and hydrogen peroxide which are toxic.

2. Diselenide, i.e., selenocystine and selenocystamine in the presence of GSH and other thiols, are reduced to selenols (RSeH), which are catalytic, they produce superoxide and hydrogen peroxide which are toxic.

3. Selenium compounds that do not react with thiols, i.e., selenate and all tested selenoethers, do not produce superoxide or hydrogen peroxide in vitro and are not toxic per se.

4. Selenate and selenoethers are toxic in tissue culture or in vivo only after being reduced to selenite or a selenoi.

5. Selenium toxicity manifests itself acutely or chronically when oxidative damage exceeds antioxidant defense or the ability of either plants or animals to form selenoproteins, selenoethers, or elemental Se (Se⁰).

1.6.1 Swine

Acute Se toxicosis in young pigs can result in clinical selenosis disease symptoms similar to those for lambs and calves (Shortridge et al., 1971).

Chronic Se poisoning in pigs is recognized by dullness, lack of vitality, emaciation, roughness of hair coat, loss of hair, soreness and sloughing of hooves, stiffness and lameness due to erosion of the joints of long bones, atrophy of the heart, cirrhosis of the liver and anemia (Underwood, 1977).

Moxon and Mahan (1981) reported that 5 ppm of Se in corn-soybean meal diets reduced the growth and feed intake of weanling swine. Growth rate was the most sensitive indicator of
chronic selenosis in swine (Goehring et al., 1984b). Goehring et al. (1984a) did not observe any effect on performance of pigs fed wheat diets containing up to 8.3 ppm Se. They further reported (Goehring et al., 1984b) that hoof lesions developed when 12 ppm Se was provided. This observation is consistent with descriptions of hoof lesions typical of chronic selenosis (Miller and Schoening, 1938; Wahlstrom et al., 1955, 1956; Wahlstrom and Olson, 1959b; Harrison et al., 1983; Goehring et al., 1984b). Wahlstrom et al. (1984) reported separation of the hooves at the coronary band after approximately 4 weeks of feeding 8 ppm inorganic Se to growing pigs.

Sodium selenite administered subcutaneously to 30 to 70 kg swine at 2.0 and 1.2 mg/kg body weight was reported to cause myopathy, increased plasma glutamic oxalacetic transaminase (GOT) activity, and clinical signs of toxicosis including paresis, trembling, and ataxia (Orstadius, 1960; Diehl et al., 1975). Acute oral intoxication in swine was induced experimentally with dosages of 13 to 23 mg Se/kg body weight, as selenite, and resulted in vomiting, diarrhea, paresis, anorexia, trembling, and depression (Miller and Williams, 1940).

A difference in the susceptibility of pigs to Se toxicity has been shown to exist among pigs of different hair color (Wahlstrom et al., 1984). Red pigs fed corn-soy diets containing 5 ppm Se developed severe selenosis, while black or white pigs were only slightly affected. Wahlstrom et al. (1984) suggested that growth rate was the most sensitive index of chronic selenosis in swine and hair selenium content was not in itself a sensitive index of chronic selenosis in swine.

Experiments carried out with adult sows using sodium selenite and revealed that conception rate, litter size, and weight of piglets were reduced by feeding high dietary Se level (10 ppm) to sows (Wahlstrom and Olson, 1959a). The growth rate was more affected after weaning than during the suckling period, where the Se challenge was smaller as the Se content in the milk was low compared to the feed (Poulsen et al., 1989). The performance of weanling piglets was affected by high dietary Se resulting in reduced feed intake and rate of gain (Wahlstrom and Olson, 1959b; Goehring et al., 1984b; Mahan and Moxon, 1984). However, when gilts were fed barley-based diets with high dietary inorganic Se (up to 16 mg/kg feed), there was no detrimental effect on sow conception, the number of piglets born, mortality, and the weight of the whole litter.
at birth (Poulsen et al., 1989). There was a tendency for a lower body weight of individual piglets at birth by Se supplementation. Colostrum and milk Se content was influenced by dietary Se treatment (Poulsen et al., 1989). The only clinical observation was a circular dark band in the hoofs of some sows fed the high Se supplementation, but the locomotion of the sows and piglets were unaffected (Poulsen et al., 1989).

1.6.2 Poultry

Since the settlement of western South Dakota and northern Nebraska in the 1890s, residents of certain regions observed poor hatchability of chicken eggs (Franke et al., 1934). Embryos were found with many types of deformities. Legs, toes, wings, beaks, and eyes were often malformed, rudimentary, or entirely lacking (Franke and Tully, 1935). Disturbances in the normal processes of bone and cartilage formation were evident. Hatchlings exhibited down that appeared greasy or wiry and never became fluffy. Investigations of the disease which began in 1929 led to cooperative work with the U. S. Department of Agriculture. These studies led to the discovery of toxic levels of Se in locally grown wheat as well as in the soil upon which it was grown (Moxon, 1937). The chick embryo is very sensitive to Se poisoning because egg hatchability is reduced by high dietary Se concentrations of Se that are too low to produce selenosis symptoms in other farm animals (Rosenfeld and Beath, 1964). The toxicity of Se is affected by several factors associated with diet, gender, and previous exposure to the element (Levander, 1972). Among the diet are related factors that affect Se toxicity are level and type of protein, dietary levels of heavy metals, other trace elements, inorganic sulfur, methyl group donors, and antioxidants (Combs and Combs, 1986). By injecting selenite into the air cell of eggs before incubation, Franke et al. (1936) induced teratogenic effects on the embryos. The highest frequencies of abnormalities resulted when the Se dosage was .7 ppm of egg yolk plus egg white; however, amounts as low as .01 ppm produced some monstronsites. Embryo deformities produced by Se toxicity resemble those induced by X-rays, various chemicals, high
and low temperatures, and centrifugal force (Trelease and Beath, 1949). Latshaw (1975) reported that Se from selenite was deposited more in the yolk than in the albumen. However, Kinder et al. (1995) demonstrated that dietary Se would affect egg albumen before the yolk Se content because broiler breeder ovum requires approximately 10 days for maturation, whereas the albumen is deposited each time an egg is formed (22 to 26 hours). Within 2 months after supplementation was withdrawn, egg Se levels declined by 50% and it appeared that eggs were acting as an avenue for clearing Se from the hen’s bodies (Heinz et al., 1987; Kinder et al., 1995). Feeding 1.4 ppm Se supplementation to emus, resulted in reduced hatchability from 52% to 40% with surviving chicks having a high incidence of leg deformities (Kinder et al., 1995). Ort and Latshaw (1978) demonstrated that 5 ppm Se caused hatchability to decrease in chickens and low egg weights resulted from 7 ppm Se supplementation. Egg production was affected by 9 ppm Se and abnormal embryos occurred at 10 ppm Se in diets.

Selenium content in feathers were increased in proportional to dietary Se. Feathers from chicks fed a diet containing 8 ppm Se were found to contain 3.4 ppm Se (Mertz and Underwood, 1986).

Several studies of aquatic birds feeding in discarded, Se-containing, subsurface agricultural drainage water containing 300 μg Se/L (normal surface waters contains < 1 μg/L) found a high prevalence of embryonic death and deformity as well as death of adult birds (Ohlenfors, 1989)

1.6.3 Dairy

Early this century, Se was identified as the active component in forages that caused livestock poisoning in South Dakota. The condition had first been recognized many years earlier by Madison, an Army doctor stationed at Fort Randall in what was the Nebraska Territory (Franke, 1934). Reports over the years of Se toxicity, occurred in grazing livestock, and we have been reminded of this problem recently by the occurrence of birth defects in waterfowl hatched on the Kesterson Wildlife Refuge in California. Selenium had concentrated in the shallow ponds by
evaporation of subirrigation drainage water from the San Joaquin Valley and was the cause of the problem (Ohlendorf et al., 1986).

When steers were fed .28, and .8 mg Se/kg body weight (approximately 10 and 25 ppm, respectively) as selenomethionine or sodium selenite, alkali disease occurred at .28 and .8 mg/kg body weight in the form of selenomethionine and to .8 mg/kg body weight in the form of sodium selenite (O'Toole and Raisbeck, 1995). This results demonstrated that the toxic level of inorganic Se was 25 ppm, while was 10 ppm organic Se in ruminant. Consequently, organic Se is more toxic in ruminant compared with inorganic Se. The distinctive histological changes that developed in the hooves, particularly in stratum medium, may account for the dystrophic digital lesions in selenosis. These lesions were accompanies by mild to marked hyperplasia and parakeratosis in laminar epithelium and, to a less extent, in coronary epidermis and loss of the normal abrupt transition between stratum spinosum and stratum comeum. The predominance of epithelial changes in hooves may distinguish Se-induced lesions from those of chronic (corial) changes in addition to irregular hyperplasia of epidermal laminae (Singh et al., 1992; O'Toole and Raisbeck, 1995).
CHAPTER 2

Effects of High Dietary Levels of Organic and Inorganic Selenium on Growth Performance, and Toxicity Characteristic in Grower – Finisher Pigs

2.1 Abstract

A 2 x 4 factorial arrangement of treatments conducted in a randomized complete block design (RCB) in two replicates evaluated the effects of high dietary levels of organic and inorganic Se with grower-finisher pigs. Two sources of Se (selenite or Se-enriched yeast) were added at 5, 10, 15, 20 ppm to corn-SBM diet. A non-Se-fortified diet served as a negative control and was a ninth treatment group. Pigs were bled at periodic intervals with serum Se and GSH-Px activity measured. Ninety crossbred barrows initially weighing an average 23.5 kg BW were allotted five pigs per pen.

After 12 wk, pigs were killed with tissue and bile collected. The results demonstrated that pig performance decreased at 10 ppm inorganic Se and at 15 ppm organic Se, resulting in interaction response ($P < .01$). Hair loss occurred at 10 ppm inorganic Se and at 15 ppm organic Se after 3 wk which was more severe in white haired pigs. Hoof separation was observed after feeding the test diets for 4 wk at 5 ppm inorganic Se and at 10 ppm organic Se. Hair loss and hoof separation occurred more frequently and at low dietary Se levels in grower pigs when inorganic Se was provided. Serum GSH-Px activity was not affected by dietary Se level or Se source. Serum GOT activity at the 12 wk period and at the end of the experiment increased as dietary Se
level increased and was approximately 2 fold higher when inorganic Se was provided, resulting in an interaction response ($P < .05$). Serum Se increased from 3 wk as dietary Se levels increased and was higher when organic Se was provided ($P < .05$). Tissue Se increased as dietary Se level increased when organic Se was provided resulting in interaction response ($P < .05$). Bile Se increased as dietary Se level increased in both Se sources ($P < .01$). The content of K, Mg, Mn, Fe, and Zn in the bile increased ($P < .10$) as dietary Se level increased although mineral intake was lower as dietary Se level increased. These results suggest that feeding a diet containing 7 to 10 ppm inorganic Se (sodium selenite) or 15 ppm organic Se (Se-enriched yeast) resulted in selenosis in grower pigs.

Key Words: Selenium, Toxicity, Pigs

2.1 Introduction

Selenium toxicity (selenosis) was reported in the 19th century and termed "alkali disease" or "blind stagers" in the USA. In 1934 Se was recognized as the toxic agent (Franke, 1934). Thereafter, Moxon (1937), and Miller and Schoering (1938) reported that selenosis symptoms in swine included hair loss, cracking of hooves, and the interruption in coronary band development of the hoof. Dietary toxic levels of Se are, however, dependent upon several factors (i.e. age, time, genetics and Se source).

Palmer et al. (1983) demonstrated that rats tolerated higher levels of inorganic Se when wheat-based diets were fed compared with than when corn-based diets. Dietary ingestion of organic Se from grain or selenomethionine was, however, less toxic in rats than inorganic Se (McAdam and Levander, 1987).

Young animals tend to more susceptible to selenosis at lower dietary Se levels than older animals (Halverson et al., 1966). Moxon and Mahan (1981) demonstrated that 5 ug Se/g diet in
corn-SBM mixture reduced the growth rate and feed intake of weanling swine. Wahlstrom et al. (1955) and Wahlstrom and Olson (1959b) demonstrated that diets above 7 ppm inorganic Se produced toxic symptoms and reduced growth performance in the growing pig.

Selenosis can be produced in either an acute or chronic form. An accidental over-supplementation or one high intake of Se can result in respiratory distress, ataxia, diarrhea, and death. Chronic selenosis results from the long-term consumption of lower levels of Se in the diet containing 5 to 20 mg/kg of Se (NRC, 1984). Growth rate appeared to be the most sensitive indicator of chronic selenosis (Goehring et al., 1984b). Toxic symptoms of chronic selenosis in swine are animal dullness, roughness of hair coat, hair loss, sloughing of hooves, cirrhosis of the liver and anemia (Ekermans and Schneider, 1982).

Most experiments which have studied Se toxicity have generally evaluated the inorganic Se source. Consequently, most of the designated toxic levels of Se are based upon those studies which fed inorganic Se forms. The objectives of this study were, therefore to evaluate high dietary levels of both organic and inorganic Se sources in growing pigs and to evaluate the selenosis tissue Se and blood Se concentrations for both Se sources.

2.3 Materials and Methods

**Treatments**: A 2 x 4 factorial arrangement of treatments conducted in a randomized complete block (RCB) design in two replicates evaluated various dietary Se levels provided as organic or inorganic Se in grower-finisher pigs. The first factor evaluated four dietary levels of added Se (5, 10, 15 and 20 ppm) from two Se sources. Sodium selenite was the inorganic source and a Se-enriched yeast provided the organic Se source. A non Se-fortified basal diet served as the ninth treatment group and was considered the negative control. A total of 90 crossbred ([Yorkshire x Landrace] x Duroc) barrows weighing an average 23.5 kg BW were allotted to dietary treatments on the basis of weight and litter. Both Se sources were premixed
in finely ground corn, analyzed for Se, and added to the diet at the appropriate treatment level. Final diet mixtures were subsequently analyzed for Se. Treatment diets contained a corn-soybean meal mixtures formulated to provide .80% lysine for grower pigs, whereas finisher pig diets contained .70% lysine. Other dietary nutrients met or exceeded NRC (1988) standards. The composition of the basal diets fed during the growing and finishing periods are presented in Table 4.

**Procedure:** Pigs were housed in complete confinement facilities with partially slotted (40%) concrete floors in groups of five per pen. Treatment diets were provided on an ad libitum basis throughout the experiment. The grower diets were fed until an average replicate pig weight of 63.5 kg BW whereupon finisher diets were provided. Performance data (weight gain and feed intake) were evaluated for a 12 wk experimental period.

Blood samples were collected from the anterior vena cava from all pigs in each replicate at 3 wk intervals. Serum was saved, frozen, and later analyzed for GSH-Px activity, serum glutamic-oxalacetic transaminase (SGOT) activities, and Se concentration. Hair loss (alopecia), disconnection of coronary band in hoof, and gait (staggers) were subjectively evaluated on each pig during each weight period.

At 12 wks, the pigs were stunned by electrocution, killed by exsanguination and samples of liver, loin, lung, pancreas, spleen, heart, kidney were collected, frozen, and later analyzed for Se. Bile was collected from the gall bladder of each pig, sealed with parafilm to avoid evaporation of moisture, stored in the refrigerator and later analyzed for Se and mineral contents.

**Analytical Methods:** Serum, feed and tissue Se analyses were conducted according to the fluorometric method (AOAC, 1995). The kidney cortex was analyzed to insure a more constant Se concentration. Serum GSH-Px activities were determined by the coupled method of Lawrence and Burk (1976). Serum glutamic-oxalacetic transaminase (SGOT) activities were determined by the method outlined by Sigma Chemical Co. (1997) with values expressed as Sigma-Frankel (SF) units/ml. Minerals were analyzed by the Inductively Coupled Photometer (ICP) method (AOAC, 1995).
Statistical analyses were performed using the GLM procedure of SAS (1985) using a RCB design. The average pig serum and tissue Se concentrations, and GSH-Px activities were averaged by pen, with pen considered as the experimental unit. Selenium source effects were contrasted by single df contrasts and the dietary level response was evaluated by linear regression. Least square treatment means are presented in the tables and appropriate interactions discussed in the text when appropriate.

2.4 Results

**Growth Performance:** High dietary Se levels of both organic and inorganic sources produced detrimental effects on performance responses in grower-finisher pigs (Table 5). Daily feed intake and daily gain decreased at the 10 ppm Se level when inorganic Se was fed but at the 15 ppm Se level when organic Se was provided. This resulted in a Se source x Se level interaction response ($P < .01$). When 15 or 20 ppm inorganic Se was provided, feed refusal occurred and feed wastage occurred within the first week of the trial. However, daily feed intake decreased more gradually than daily gain when the dietary Se level increased from feeding organic Se, but more so at the 20 ppm organic Se level. Final body weight began to decrease at 10 ppm inorganic Se and was only lowered at 20 ppm Se when organic Se was fed. This resulted in an interaction response ($P < .05$).

**Selenium Toxicity Symptoms:** Rough hair coat and less pig activity was observed when 15 or 20 ppm inorganic Se was provided. A thin dorsal body was observed at 15 and 20 ppm inorganic Se within after 2 wk of starting the experiment. Starving the relatively rapid response to high dietary levels of inorganic Se. The lethargic weakened condition was attributed to the lower energy consumption. Hair loss (alopecia) occurred at 10 ppm inorganic Se and 15 ppm organic Se within 3 wk of the start of the trial. This response was particularly noticeable in white haired pigs.
Hoof separation was observed after 4 wk around the coronary band at >5 ppm inorganic Se but at 10 ppm when organic Se was fed. Colored haired pigs (red or black) were heavier than white haired pigs in both Se source but there was no difference in body weights between red and black haired pigs (Figure 5). Anterior paralysis and (or) weakness was observed at 20 ppm Se in both sources (Figure 6). After the animals were killed at 12 wk, white spots in the liver attributed to fatty infiltration and white band at the edge of liver lobes were observed when 15 and 20 ppm inorganic Se was provided.

**Blood Measurements:** Serum Se concentrations of pigs increased as dietary Se level increased ($P < .01$) and was higher when organic Se was provided ($P < .05$; Table 6). There was a tendency for serum Se to decline from 9 to 12 wk, particularly when the higher dietary Se concentrations of both Se sources were fed. This was attributed to the lower feed intake at the higher Se levels.

Serum GSH-Px activity increased as dietary Se level increased ($P < .01$) and tended to be somewhat higher when inorganic Se was provided but the interaction response was not significant ($P > .15$) except at the 12 wk period.

Serum GOT activities at 12 wk increased as dietary Se level increased ($P < .01$) and was approximately 2 fold higher when the inorganic Se source was provided compared with organic Se treatment groups. This resulted in interaction response ($P < .05$).

**Tissue Measurements:** Tissue Se contents were higher ($P < .01$) as the dietary Se level increased when either dietary Se source was fed (Table 6). When the basal diet was fed, the kidney had the highest tissue Se content followed by the pancreas, spleen, liver, heart, lung and loin. This same order in Se concentration resulted when organic or inorganic Se was fed. Kidney Se content increased as dietary Se level increased and was higher when pigs were fed organic Se. This resulted in an interaction response ($P < .05$). Liver Se increase to a higher concentration than kidney as the dietary Se level increased particular when organic Se was provided. Selenium concentrations in the lung, pancreas, spleen, increased and were 3 to 5 fold higher when pigs were fed organic Se. This resulted in an interaction response ($P < .01$).
Selenium concentration in the loin and heart muscle was lower than other internal organs when inorganic Se was provided. Heart Se was relatively constant when inorganic Se was provided but increased linearly ($P < .01$) as dietary Se level increased when organic Se was fed. Loin Se concentration increased as dietary Se level increased ($P < .01$) and increased more when organic Se was provided ($P < .01$). This resulted in an interaction response ($P < .05$). When inorganic Se was fed, loin Se content was approximately two fold higher at 5 ppm than the basal diet but was similar between 5 to 20 ppm Se.

**Bile minerals:** The Se concentration in bile increased linearly ($P < .01$) as dietary Se level increased when either Se source was fed (Table 7). The lower bile Se concentration at 20 ppm Se from the inorganic Se group was attributed to their lower feed intake (Table 5). The elements, Na, K, Mg, Mn, Fe, and Zn concentration increased to 15 ppm inorganic Se level even though feed intake had decreased whereas no increase resulted when organic Se was fed. Zinc concentration in the bile remained high although Zn intake decreased as dietary Se level increased. Copper content of bile was not affected by dietary Se level or Se source.

Bile color of fed the basal diet was yellow but became extremely dark when pigs were fed >10 ppm inorganic Se and 20 ppm organic Se (Figure 7). This was attributed to hepatic degeneration at high dietary Se levels.

### 2.5 Discussion

Previous research indicated reduced pig performance after supplementing of 4 to 8 ppm inorganic Se to weanling (Moxon and Mahan, 1981) and grower-finisher pigs (Goehring et al., 1984a,b; Wahlstrom et al., 1984). Consequently, the toxic level of Se has been established at 4 to 5 ppm by the National Research Council (1983). Wahlstrom et al. (1984) demonstrated that pig growth rate was the most sensitive indicator for chronic Se toxicity. In this experiment, feed
intake and feed refusal also indicated Se toxicity. Pigs consumed lower amounts of feed (energy) in the high Se treatment group which resulted in the lower body weights. Final body weight, daily gain, and daily feed intake thus declined from 10 ppm inorganic Se and 15 ppm organic Se.

Se toxicity symptoms of hair loss and hoof separation were observed after approximately 4 wk of feeding the high Se diets. This occurred at a lower Se level when inorganic Se compared to when organic Se was fed. Goehring et al. (1984b) and Wahlstrom et al. (1984) also reported that hoof lesions occurred after approximately 4 wk exposure to high Se diets. Serum Se concentration plateaued between 15 and 20 ppm inorganic Se level and may be explained by the lowered feed intake when toxic Se levels were consumed.

Paralysis or weakened leg was observed at 20 ppm Se when either Se source was fed at the 12 wk period. This condition has been reported previously when 20 ppm Se was fed (Goehring et al., 1984b; Harrison et al., 1983) and at 50 ppm Se (Wilson et al., 1982) in growing pigs.

Hair loss in weaning pigs resulted when hair Se contents were at 1.5 to 2.0 ppm Se when 7 ppm inorganic Se and at 10 ppm Se when 10 ppm organic Se was fed, respectively (Chapter 3). This suggests that hair Se concentration might be a sensitive indicator of chronic Se toxicity in swine. Olson et al. (1954) demonstrated that hair Se from a dietary Se concentration of 5 to 10 ppm caused a potential chronic selenosis problem and chronic selenosis could be expected when hair Se concentrations were over 10 ppm.

A difference in susceptibility to Se toxicity occurred among pigs of different hair colors. White haired pigs seemed to be more sensitive to Se toxicity compared with colored hair (red or black) pigs. The body weight of the white haired pigs was lower than the colored haired pigs. We had also documented that hair loss in weaning pigs was also more severe in white haired pigs even though hair Se was at a higher concentration in colored rather than white hair (Chapter 3). Hoof separation was also more frequent in white haired pigs. In contrast, Wahlstrom et al. (1984) demonstrated that black and white pigs were less affected by Se toxicity than red haired pigs.
Serum GSH-Px activities increased as the dietary Se level increased in both Se sources. Serum GSH-Px activity is considered a sensitive indicator of Se status when low dietary levels of Se are fed (Thomson and Fraser, 1983) but does not seem to be a good indicator when excess dietary Se is consumed (Goehring et al., 1984a; Mahan and Parrett, 1996; Kim and Mahan, 1999).

Serum GOT (SGOT) activity can reflect cellular damage because it is a cytosolic enzyme which is released into the circulatory fluid when cell integrity is insulted. Serum GOT activities at the end of experiment increased as dietary Se level increased and were approximately 2 fold higher when pigs were fed inorganic Se. This suggests that cellular damage increased as dietary Se level increased particularly when inorganic Se was fed.

Tissue Se increased as dietary Se level increased and was highest when organic Se was provided. Loin and heart Se concentrations were maintained at a constant concentration when the various dietary Se levels of inorganic Se was fed but increased when organic Se was provided. Lung Se was approximate three to four fold higher than loin Se as dietary Se level increased when pigs were fed inorganic Se. These results indicate that inorganic Se was not retained in many body tissues when pigs were fed inorganic Se compared with pigs fed organic Se. Selenium can be excreted through the lung when high levels of inorganic Se are provided (Hirooka and Galambos, 1966; Diplock, 1976; Ganther, 1986). Exhalation of the volatile Se metabolite, dimethyl selenide, is indicative of acute toxicity and exhaled in proportion to the amount of Se administered (Medinsky et al., 1981).

When organic Se was fed, the Se concentrations in all tissues increased and muscle tissue Se concentration such as loin, heart also increased as dietary Se level increased. Loin and other muscle tissue Se, represents stored Se which is not readily available for metabolic assimilation unless tissue catabolism takes place (Mahan and Kim, 1996).

Bile is considered to have a minor route in Se excretion (Levander and Bauman, 1966) but in the present experiment Se concentrations in bile increased as dietary Se level increased particularly when inorganic Se was fed.
Biliary micro-mineral contents (Zn, Fe, Mg) increased as the dietary Se level increased even though these mineral intakes were lower from the reduced feed intakes of higher Se levels. This suggests that an increase in the excretion of several essential micro-mineral may occur and precipitate other deficiency. Liver, spleen, and bone marrow could subsequently be damaged by high dietary Se. When protein and iron are released from Hb during Hb destruction, a green pigment, biliverdin is converted to a yellow color in reticuloendothelial cells of the liver, spleen and bone marrow (Murray et al., 1996). Because of disfunctioned organs, intact biliverdin would be transported to the liver and passed to the gallbladder. Consequently the dark color in the bile was more apparent rather than the yellow color. The dark color of bile at the high levels of Se thus suggests that tissue damage probably occurred.

2.6 Implications

Feeding a diet containing 5 to 10 ppm Se of sodium selenite or 15 ppm of organic Se (Se-enriched yeast) resulted in selenosis condition and was considered toxic for grower-.finisher pigs. Serum and tissue Se content increased as dietary Se level increased but increased more when organic Se was fed. Colored hair pigs (red or black) appeared to be more resistant to Se toxicity compared to white color pigs. The excretion of essential micro-mineral such as Mn, Zn, through the biliary tract increased was higher as dietary Se level increased when pigs were fed inorganic Se.
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Grower</th>
<th>Finisher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>79.15</td>
<td>82.95</td>
</tr>
<tr>
<td>Soybean meal, 44% CP</td>
<td>18.00</td>
<td>14.50</td>
</tr>
<tr>
<td>L-Lysine • HCl</td>
<td>.10</td>
<td>.10</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
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<td>.95</td>
</tr>
<tr>
<td>Limestone</td>
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<td>.05</td>
<td>.05</td>
</tr>
<tr>
<td>Salt</td>
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<td>.45</td>
</tr>
<tr>
<td>Se premix</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Vitamin mix e</td>
<td>.20</td>
<td>.20</td>
</tr>
<tr>
<td>Antibiotic f</td>
<td>.10</td>
<td>.05</td>
</tr>
</tbody>
</table>

**a** Inorganic or organic Se was premixed in ground corn and added to the diet at the appropriate treatment levels.

**b** Formulated to .80% lysine, .65% Ca and .55% P.

**c** Formulated to .70% lysine, .55% Ca and .50% P.

**d** Supplied per kilogram diet: 10 mg of Cu (copper oxide); 100 mg of Fe (ferrous sulfate); .2 mg of I (calcium iodate); 40 mg of Mn (manganese oxide); 120 mg of Zn (zinc oxide).

**e** Supplied per kilogram diet: 1,750 IU of vitamin A; 200 IU of vit D3; 11 IU of vitamin E; .5 mg of vitamin K; 3.0 mg of riboflavin; 10 mg of pantothenic acid; 13 mg of niacin; .3 mg of folacin; .05 mg of biotin; 15 ug of vitamin B12; .4 g of choline and 66 mg of BHT.

**f** Tylan was added at 22 and 10 mg per kilogram diet for the grower and finisher diets, respectively.

Table 4. Percentage composition of experimental diets (as fed)
<table>
<thead>
<tr>
<th>Item</th>
<th>Basal</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>SEM</th>
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<td>10</td>
<td>10</td>
<td>10</td>
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<td>Weight, kg</td>
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<td></td>
</tr>
<tr>
<td>Initial</td>
<td>24.86</td>
<td>24.50</td>
<td>24.54</td>
<td>24.81</td>
<td>24.30</td>
<td>24.36</td>
<td>25.43</td>
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<td>84.46</td>
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<td>85.09</td>
<td>82.71</td>
<td>82.96</td>
<td>68.91</td>
<td>5.20&lt;sup&gt;abc&lt;/sup&gt;</td>
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<tr>
<td>Daily gain, kg</td>
<td>.84</td>
<td>.82</td>
<td>.66</td>
<td>.25</td>
<td>.08</td>
<td>.83</td>
<td>.78</td>
<td>.71</td>
<td>.55</td>
<td>.04&lt;sup&gt;abd&lt;/sup&gt;</td>
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<tr>
<td>Daily feed intake, kg</td>
<td>2.38</td>
<td>2.31</td>
<td>1.89</td>
<td>1.10</td>
<td>.66</td>
<td>2.38</td>
<td>2.25</td>
<td>2.03</td>
<td>1.70</td>
<td>.12&lt;sup&gt;abd&lt;/sup&gt;</td>
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<tr>
<td>Gain : feed</td>
<td>.35</td>
<td>.35</td>
<td>.35</td>
<td>.23</td>
<td>.12</td>
<td>.35</td>
<td>.35</td>
<td>.35</td>
<td>.32</td>
<td>.01&lt;sup&gt;abd&lt;/sup&gt;</td>
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</table>

<sup>a</sup> Dietary Se source response (P < .01).
<sup>b</sup> Dietary Se level linear response (P < .01).
<sup>c</sup> Dietary Se level x Se source response (P < .05).
<sup>d</sup> Dietary Se level x Se source response (P < .01).

Table 5. Treatment effects of dietary selenium level and selenium source on growth performance of grower-finisher pigs.
<table>
<thead>
<tr>
<th>Item</th>
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<th>5</th>
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<th>10</th>
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<td>1.767</td>
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<td>1.279</td>
<td>2.352</td>
<td>2.828</td>
<td>.25f</td>
</tr>
<tr>
<td>6 wk</td>
<td>.136</td>
<td>.775</td>
<td>1.169</td>
<td>1.775</td>
<td>1.779</td>
<td>.866</td>
<td>1.353</td>
<td>2.769</td>
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<tr>
<td>9 wk</td>
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<td>.780</td>
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<td>2.163</td>
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<td>.951</td>
<td>1.649</td>
<td>2.945</td>
<td>3.337</td>
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<td>12 wk</td>
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<td>.662</td>
<td>1.096</td>
<td>1.886</td>
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<td>.778</td>
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<td>.255</td>
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<td>.461</td>
<td>.413</td>
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<td>.03f</td>
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<td>.524</td>
<td>.551</td>
<td>.715</td>
<td>.773</td>
<td>.524</td>
<td>.557</td>
<td>.556</td>
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<td>9 wk</td>
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<td>.826</td>
<td>.516</td>
<td>.604</td>
<td>.588</td>
<td>.675</td>
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</tr>
<tr>
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<td>.564</td>
<td>.559</td>
<td>.827</td>
<td>.485</td>
<td>.525</td>
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<sup>a</sup> One unit of GSH-Px activity equals 1 umole of NADPH oxidized per minute/mL serum.
<sup>b</sup> One Sigma-Frankel [SF] unit of GOT will form 4.82 x 10<sup>-4</sup> umole glutamic acid/min at pH 7.5 and 25 °C.
<sup>c</sup> Samples were pooled by treatment in each replicate.
<sup>d</sup> Dietary Se source response (P < .05).
<sup>e</sup> Dietary Se source response (P < .01).
<sup>f</sup> Dietary Se level linear response (P < .01).
<sup>g</sup> Dietary Se level x Se source response (P < .05).
<sup>h</sup> Dietary Se level x Se source response (P < .01).

Table 6. Treatment effects of dietary selenium level and selenium source on serum and tissue Se of grower-finisher pigs.
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<th>10</th>
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<td>10</td>
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<td>10</td>
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*a* Dietary Se source response (P < .01).
*b* Dietary Se level linear response (P < .05).
*c* Dietary Se level linear response (P < .01).

Table 7. Treatment effects of dietary selenium level and selenium source on mineral concentrations in bile of grower-finisher pigs.
Figure 5. Relative body size of pigs of different hair colors after feeding 10 ppm inorganic Se for 12 weeks (left = white, center = black, right = red pig)

Figure 6. Anterior paralysis of pig (right) after feeding 20 ppm inorganic Se for 12 weeks
Figure 7. Darkened bile after feeding different dietary Se levels and Se sources for a 12 week period (1 = basal, 2 = 10 ppm inorganic Se, 3 = 20 ppm inorganic Se, 4 = 10 ppm organic Se, 5 = 20 ppm organic Se treatment)
CHAPTER 3

Prolonged Feeding of High Dietary Levels of Organic and Inorganic Selenium to Swine from 25 kg Body Weight through One Parity to Primiparous Sows

3.1 Abstract

An experiment was conducted to evaluate the effects of high dietary Se levels from organic and inorganic Se sources on resultant growth performance, liver and serum Se concentrations in grower-finisher pigs. The females were subsequently continued on their treatment diets through a complete reproductive cycle to evaluate the possible detrimental effects on reproduction and her progeny. The experiment was conducted in a $2 \times 4$ factorial arrangement of treatments in a randomized complete block (RCB) design. Both Se sources were added at 3, 7, 10 and 10 ppm Se. A total of 88 crossbred pigs were fed their treatment diets from 25 to 105 kg BW in two replicates. Blood was collected at periodic intervals with serum Se and GSH-Px activity determined. Liver tissue was collected from 4 animal after a 14 wk period for Se analysis. Growth performance during the grower period decreased as dietary Se level increased in both Se sources which resulted in an interaction response ($P < .01$). Serum Se and liver Se increased as dietary Se level increased and were higher when organic Se was provided resulting in an interaction response ($P < .01$). Hoof separation in some grower-finisher pigs occurred at 7 and 10 ppm inorganic Se but toxic symptoms of Se were not observed in the organic Se treatment
groups. A total of 22 gilts were obtained from the above study and continued on their treatment diets. Gilts were subsequently bled at periodic intervals with serum Se and GSH-Px activity measured. Colostrum and milk were collected daily from 1 to 7 day postpartum and at 10, and 14 day postpartum (weaning) for Se and protein analysis. Pig tissue (3/treatment) was collected prior to colostrum consumption and at weaning. Pig blood was collected at 0, 7, 14 and at 49 day of age. The results suggested that reproductive performance tended to decline ($P > .15$) as Se level increased particularly when organic Se was provided. Litter gain decreased as dietary Se level increased ($P < .05$) when sows were fed the organic Se source. Colostrum and milk Se increased as dietary Se level increased and was 2 to 5 fold higher when sows were fed organic Se source, which resulted in an interaction response ($P < .01$). Neonatal pig tissue Se and serum Se increased as dietary Se level increased when organic Se was provided, resulting in an interaction response ($P < .01$). Neither Se source nor Se level affected serum GSH-Px activity in the sow, neonate or weaned pig. Weanling pig tissue Se increased as dietary Se level increased when organic Se source was fed to the sow, which resulted in an interaction response ($P < .01$). Hoof separation and hair loss in pigs occurred above 7 ppm Se when sows were fed inorganic Se, but when organic Se was fed, hair loss was observed at 10 ppm Se. Serum Se in all pigs returned to similar values by 49 day of age after pigs were fed postweaning diets that contained .3 ppm Se (inorganic Se). These results suggest that Se transfer from the dam to the litter was higher during gestation and lactation when sows were fed organic Se and increased as Se level increased. The inorganic form seemed to be more toxic at lower dietary levels in nursing pigs whereas reproductive performance of sows was reduced when 10 ppm organic Se was fed.

Key words: Selenium, Selenosis, Reproduction, Pigs
3.2 Introduction

High dietary selenium (Se) levels have resulted in growth depression, hoof separation and hair loss (alopecia) when grower pigs were fed 5 to 8 ppm Se as sodium selenite (Goehring et al., 1984a,b; Wahlstrom et al., 1984). Our previous research demonstrated that a diet containing 10 ppm inorganic Se (sodium selenite) or 15 ppm organic Se (Se-enriched yeast) produced toxic symptoms in grower pigs (Chapter 2). The reason for the different responses between the two Se sources is attributed to the seleno amino acids (selenomethionine, selenocysteine, etc.) from the organic Se source which are retained at higher concentration in muscle and other body proteinaceous tissue (Mahan and Parrett, 1996). Consequently less Se from organic Se is available to tissues where toxic responses are demonstrated.

When organic Se was fed at high dietary levels, tissue Se concentrations are higher in the liver, pancreas, and kidney compared with pigs fed inorganic Se (Mahan and Kim, 1996; Mahan and Parrett, 1996; Chapter 2). When replacement gilts are fed organic Se, the effect of these higher accumulated Se depots in various tissues may, however, ultimately be detrimental on subsequent reproductive responses. Wahlstrom and Olson (1959a) had previously demonstrated that conception rate, litter size and weight of piglets at birth were reduced when sows were fed 10 ppm sodium selenite from 15 kg body weight through the reproductive period. In contrast, Poulsen et al. (1989) reported no effect on sow conception rate and litter size when a barley-soybean meal diet containing 16 ppm Se as sodium selenite was fed only during gestation and lactation but the weight of weaning pigs decreased. Because organic Se can effectively replace inorganic Se in the production of GSH-Px, and is also retained at a higher concentration in all tissues, we were interested in evaluating the long term effects of high dietary levels of organic and inorganic Se sources fed to pigs from 25 kg body weight and continuing through a complete reproductive cycle. Sow performance, tissue Se concentrations and toxic symptoms of Se in both the sow and progeny were subsequently evaluated.
3.3 Materials and Methods

**General.** An experiment was conducted to evaluate the effects of various levels of organic and inorganic Se sources on growth performance during the grower-finisher period. The gilts from this study were continued on their dietary Se treatments through a complete reproductive cycle to evaluate the long-term carry-over effect.

**Growing-Finishing Period.** The grower-finisher experiment was a $2 \times 4$ factorial arrangement of treatments conducted in a randomized complete block design (RCB) in two replicates. The first factor was Se source (organic vs. inorganic) and the second factor was the dietary level of added Se (.3, 3, 7, 10 ppm). Sodium selenite was the inorganic and a Se-enriched yeast source, largely comprised of selenomethionine, served as the organic Se source.

A total of 88 crossbred ([Yorkshire x Landrace] x Duroc) pigs (24 barrows and 64 gilts) weighing an average 25 kg BW were allotted to the eight dietary treatments on the basis of weight, sex, and litter.

Both Se sources were premixed to 200 mg/kg concentration in finely ground corn, and added to diets at the appropriate treatment level. Both the premix and final diet mixtures were subsequently analyzed for their Se concentrations. Treatment diets for the grower period contained a corn-soybean meal mixture formulated to .80% lysine; the finisher diets were formulated to .75% lysine. Other dietary nutrients met or exceeded the NRC (1988) standards.

Growing-finisher pigs were housed in groups of five to six animals per pen in complete confinement facilities with partially slotted (40%) concrete floors but with an equalized number of pigs per pen within replicate. Treatment diets were provided on an ad libitum basis until 105 kg body weight. Individual pig blood samples were collected from the anterior vena cava initially and at 6 and 14 wk after being fed their test diet. After 14 wk, a total of four to five pigs from
each treatment group were electrically stunned, killed by exanguination and a liver sample collected, frozen and later analyzed for Se.

**Gestation:** A total of 22 gilts were obtained from the grower-finisher experiment at approximately 105 kg BW but continued on their finisher diet to 115 kg body weight. Gilts were placed in individual crates and fed the finisher treatment diet once daily to attain 135 kg by 8 mo. of age. At 8 mo. of age the gilts were artificially inseminated twice at 12 hr intervals. Gestation diets were formulated to .75% lysine, .90% Ca, and .70% P. Gilts were fed their treatment diets at 1.95 kg daily from breeding to 109 day postcoitum.

Blood samples were collected from each gilt from the anterior vena cava at breeding and 109 day postcoitum, serum saved, frozen and later analyzed for GSH-Px activity and Se concentration. Gilts were weighed and backfat measurements collected at the last rib (Renco Lean-Meater, Minneapolis, MN) at breeding and 109 day postcoitum.

**Lactation:** At 109 day postcoitum, pregnant gilts were placed into rubber coated expanded metal floor individual farrowing crates and fed their lactation treatment diet on an ad libitum basis to weaning (14 day). Lactation diets were a corn-soybean meal- L-lysine-HCl mixture formulated to provide .90% lysine, .90% Ca, and .70% P. Other nutrients provided in these diets met or exceeded NRC (1988) standards. The composition of basal diets fed during the grower, finisher, gestation and lactation periods are presented in Table 8.

Upon farrowing, three to four pigs per treatment group were killed prior to colostrum consumption and the liver, loin, pancreas, kidney, hoof and hair samples collected, frozen and later analyzed for Se. Approximately 10 ml of colostrum and milk were collected from the functional glands of each sow daily from parturition to 7 day, and on day 10 and 14 postpartum, frozen and later analyzed for Se and milk protein.

Hemoglobin (Hb) and packed cell volume (PCV) were measured because previous research had demonstrated that Hb level was lower in chronic selenosis of rats (Franke and Potter, 1934; Halverson et al., 1966) and dogs (Moxon, 1937). Reticulocytes were measured because
immature erythroid cellular elements were found to increase in Se deficient chicks (Bartholomew et al., 1998).

Sows were bled at 7 and 14 day postpartum from the anterior vena cava and three pigs from each litter were also bled via cardiac puncture prior to colostrum consumption and at 7 and 14 day of age for Hb and PCV analysis before centrifugation. Bloods was centrifuged (4°C), serum saved and frozen for later Se analysis and measurement of GSH-Px activity.

At weaning (14 day), three to four pigs from each sow treatment group were stunned by electrocution and killed by exsanguination. Liver, loin, lung, pancreas, kidney, hoof, hair samples were collected from each pig, frozen and later analyzed for Se. Sows upon weaning were stunned by electrocution, killed by exsanguination and samples of liver, loin, lung, pancreas, spleen, kidney, hoof, hair collected and frozen for later Se analysis.

**Analytical Methods:** Colostrum, milk, serum, feed, and tissue Se analyses were conducted according to the fluorometric method outlined by AOAC (1995). The kidney, cortex was analyzed because Hoffman et al. (1973) had demonstrated that the Se concentration from this area was more uniform. Hemoglobin (Hb) analysis was conducted colorimetrically with Hb kits (Sigma, No. 525) by using cyanmethemoglobin and drabkin’s solution. Packed Cell Volume (PCV) analysis was conducted using a micro-capillary centrifuge (International Equipment Co., Boston, MA) and expressed as percentage of packed red blood cells in whole blood. Reticulocyte analysis was measured by the stained method of Williams et al. (1990).

Serum GSH-Px activities were determined by the coupled method of Lawrence and Burk (1976). Hair samples were collected from the dorsal-midline of all pigs, washed three times with 60°C distilled water, dried and analyzed for Se.

Statistical analyses of the grower pig data were conducted using the GLM procedure of SAS (1985) in a RCB design. The reproductive data were analyzed as a CRD design. The individual sow and litter was considered the experimental unit. Main effects were contrasted either by single df comparisons or by linear regression for dietary level response.
3.4 Results

Grower Period: The effect of Se source and Se level on daily gain, feed intake, and gain to feed ratio decreased ($P < .05$) as the dietary Se level increased for both organic and inorganic Se sources (Table 9).

Serum Se increased at the 6 and 14 wk period as dietary Se level increased and was higher at each measurement period when pigs were fed organic Se. This resulted in an interaction response ($P < .01$). Liver Se concentration increased as dietary Se level increased and was higher when organic Se was the Se source, particularly at higher dietary levels resulting in an interaction response ($P < .01$).

Hoof lesions occurred when pigs were fed > 7 ppm of inorganic Se and at 10 ppm of organic Se after 4 wk of the trial. These pigs were not used in the reproductive study. Although hair loss (alopecia) did not occur in this experiment, a rough hair appearance resulted when 10 ppm Se was provided.

Sow Reproductive Performance: The number of sows available and which subsequently farrowed for this experiment was low for all treatment groups, which subsequently affected the statistical inferences. Consequently, reproductive performance responses demonstrated that dietary Se level or Se source did not significantly ($P > .15$) influence sow body weights, weight changes or backfat thickness during gestation and lactation (Table 10). Backfat thickness at 110 day postcoitum declined when Se levels increased in both Se sources, but the decrease was consistently lower when sodium selenite was provided. This resulted in an interaction response ($P < .01$). Sow lactation feed intake tended to be lower at the higher Se level ($P < .17$).

The number of pigs born (total, live, stillborn) were not affected by dietary Se level or Se source (Table 10). Litter and individual pig birth weights decreased as dietary Se level increased and were lower when organic Se was fed to the sow. This resulted in an interaction response ($P < .05$). Litter gain from 0 to 14 day postpartum decreased as Se level increased ($P < .05$)
particularly in the organic Se treatment group. The number and percentage of pigs weaned declined ($P < .15$) as dietary Se level increased and was lower when the organic Se source was fed.

**Sow Blood Measurements:** Sow serum Se concentrations increased at each measurement period as dietary Se level increased and was highest when organic Se was provided. This resulted in an interaction response ($P < .01$; Table 11). Serum Se concentrations of gilts decreased in all treatment groups from breeding to 110 day postcoitum when inorganic Se was fed, whereas it was similar or higher when organic Se was fed. Feed intake had been changed from an ad libitum basis to 1.95 kg/day from the finisher to gestation periods, respectively. Consequently, the organic Se in the body tissue had accumulated during finisher period and was a source of Se for the blood fluids in the organic Se fed group as tissue catabolism took place. After farrowing, serum Se concentrations increased during lactation when inorganic Se or organic Se sources were fed which was attributable to the higher feed intakes (Table 11).

Gilt hemoglobin (Hb) concentrations and Packed Cell Volume (PCV) were not affected by Se source. The number of red blood cells and percentage of reticulocytes were not affected by dietary Se levels or Se source.

Serum GSH-Px activities were consistently lower at each measurement period when .3 ppm Se from the inorganic Se was compared with the organic Se fed group and was lower at 110 day postcoitum for all treatment groups. The activity of serum GSH-Px was not affected by dietary Se source or Se level (Table 11). At weaning, sow serum GSH-Px activities were higher than at 110 day postcoitum and increased as the dietary Se level increased when inorganic Se was provided. This resulted in an interaction response ($P < .05$).

**Colostrum and Milk Composition:** Colostrum Se concentrations were highest from 0 to 2 day postpartum higher as dietary Se level increased, and when sows were fed organic Se. This resulted in an interaction response ($P < .01$; Table 12). Colostrum Se concentrations decreased from 0 to 3 day postpartum but more rapidly in the mature milks. From 3 to 14 day postpartum
the Se content in the milks were relatively constant. Selenium content of colostrum and milk was consistently 2 to 5 fold higher when sows were fed organic Se.

Protein content of colostrum and milk were not affected by dietary Se level or Se source (Table 12). There was the expected decline in colostrum protein concentrations from parturition to 2 day postpartum with a relatively smaller decline from 3 to 14 day postpartum.

**Sow Tissue Selenium (Weaning):** Sow tissue Se concentrations at 14 day postpartum (weaning) were affected by both Se level and Se source (Table 13). Kidney Se concentration was highest followed by the liver, hoof, spleen and pancreas.

The Se concentration in the loin represents the largest total amount of Se in the body reflecting the large muscle mass of the animal. Loin Se concentrations thus increased as dietary Se level increased and were higher when gilts were fed the organic Se source which resulted in an interaction response ($P < .01$). Selenium concentrations in sow hoof, hair, lung, pancreas, spleen also increased as dietary Se level increased and when the organic Se source was fed which resulted in a Se source x Se level interaction response ($P < .01$).

**Pig Blood Measurements:** Neonatal pig serum Se was higher when the dietary Se level fed to the pregnant sow increased and when the organic Se source was fed which resulted in an interaction response ($P < .05$; Table 14). This suggests that Se was effectively transferred from the dams maternal blood supply to the fetus during pregnancy from either Se source, but more Se was transferred when organic Se was fed.

Neonatal pig hemoglobin (Hb) and packed cell volume (PCV) were not affected by dietary Se level or Se source. Serum GSH-Px activities of neonatal pigs increased ($P < .01$) as dietary Se fed to the sow increased but was not affected by dietary Se source.

Pig serum Se concentrations at 7 and 14 day of age increased as sow dietary Se level increased and was higher when organic Se was fed to the sow. This resulted in an interaction response ($P < .01$). Serum Se concentrations at 7 and 14 day were similar when the .3 ppm Se level was fed from either Se source, but increased approximately two fold over that of the
neonate. This suggests that the milk Se consumed by the pig was efficiently absorbed during the nursing period.

Pig Hb percentages were not affected by dietary Se level or Se source (Table 14). PCV decreased as dietary Se fed to the sow increased (P < .05) but was not affected by dietary Se source.

At 49 day post-weaning and after pigs had consumed a postweaning diets which contained .3 ppm inorganic Se, the resulting serum Se contents were similar for all progeny.

Sulfur (S) content in the hoof of weaning pigs increased as the dietary Se level increased when sows were fed organic Se but was not affected by dietary Se level when inorganic Se was fed.

**Neonatal Pig Tissue Selenium:** Neonatal pigs killed prior to colostrum consumption demonstrated that tissue Se concentrations was higher as the dietary Se level fed to the pregnant gilt increased and was highest when organic Se had been provided. This resulted in an interaction response (P < .01; Table 15). Liver Se concentrations had the highest Se value among tissues in all treatment groups and were two fold higher than when inorganic Se had been fed to the sows. Liver Se was similar at 7 and 10 ppm Se in both Se sources implying that Se transfer through placenta or subsequently retained in the liver may have been limited during gestation or that tissue retention of the element or amino acid had been plateaued. The loin Se of pigs from sows fed organic Se had a ten fold higher Se content than those fed inorganic Se.

**Weaning Pig Tissue Selenium:** After consuming sow colostrum and milk for a 14 day period, all weaning pig tissue Se contents increased, but the increase was higher in the pigs from fed higher dietary Se levels (Table 15). This resulted in an interaction response (P < .01). Liver followed by hoof, hair, kidney had higher Se contents regardless of Se source.

Severe hair loss (alopecia) occurred in nursing pigs by 7 day of age when sows were fed inorganic Se 7 ppm and at 10 ppm organic Se (Table 9; Fig 8). Pig hair loss initially occurred in white hair areas but then later than in the colored haired areas.

Hoof separation was observed in young pigs when sows had been fed 7 ppm inorganic Se diet but was not observed in any of the pigs from sows fed organic Se. Dark coronary bands
appeared around the hoof at the initial stage of hoof separation. Hoof separation did not occur in organic Se treatments even though hoof Se concentrations were much higher than when inorganic Se was fed to the sows.

3.5 Discussion

Previous research had indicated that weaning or growing pig performance was reduced by supplementing Se at 4 and 8 ppm when inorganic Se was used (Moxon and Mahan, 1981; Goehring et al., 1984a,b; Wahlstrom et al., 1984). Wahlstrom et al. (1984) suggested that growth rate was a sensitive index of chronic Se toxicity in growing pigs. Blood Se can be an indicator of Se intake and body status when swine are fed diets low or adequate in Se (Young et al., 1976; Chavez, 1979) and when excess dietary Se is consumed (Meyer et al., 1981; Goehring et al., 1984a). Our experiment confirmed this observation. The lower serum Se at the 14 wk period could have been the result of the lower feed intake relative to body weight particularly at the 14 wk period. Goehring et al. (1984a) had demonstrated that whole blood Se concentration increased from 6 to 17 wk when pigs were fed high Se containing diets.

The reproductive performance of sows fed high levels of inorganic Se supplementation were reported previously. Wahlstrom and Olson (1959) had previously demonstrated a lower number of live pig born when gilts were fed a corn-oat diet supplemented with sodium selenite at 10 ppm Se. In contrast, when Poulsen et al. (1989) supplemented sodium selenite as much as 16 ppm they did not show a decreased reproductive performance when primiparous sows were fed a barley and soybean meal diet during gestation and lactation. In the present experiment our results indicated that the number of pigs born, litter birth weights and litter gains during lactation tended to decrease as dietary Se level increased but particularly when organic Se had been fed. This implies that the higher quantities of Se which had accumulated in the tissues of sows, which
had been fed organic Se, may have caused this response. Consequently, accumulated Se may have a detrimental effect on sow reproductive performance if fed over a prolonged period. The accumulated Se in sow body tissues would subsequently be transferred to colostrum and milk to the nursing pig. Because the liver is a labile Se tissue, this could be the possible reason why the liver Se concentration of sows at weaning was lower than the livers of finisher pigs when they were fed inorganic Se, whereas it was higher when organic Se was fed. The increased serum Se concentration of sows after farrowing and weaning may be the result of accumulated Se but also the higher consumption of Se during the lactation compared with gestation period.

Colostrum and milk Se concentrations were elevated as dietary Se level increased but were markedly higher when the organic Se source was fed to the sow. This suggests that the excess dietary Se and that which had accumulated in muscle or other internal organs can be transferred to mammary tissue and subsequently into milk. The colostrum and milk Se values in previous research (Poulsen et al., 1989) were much lower than that obtained in our experiment. This discrepancy may be explained by differences in the length of the feeding period of the Se treatments because in the trial of Poulsen et al. (1989) gilts were fed 16 ppm inorganic Se only during the gestation period.

Milk Se contents were relatively constant from 3 day through weaning within Se treatment groups but was higher when organic Se was fed. The higher milk Se content from dams fed organic Se subsequently resulted in higher pig serum and tissue Se contents at weaning. This suggests that when higher organic Se diets are fed to sows that Se can be directly transferred to neonatal pigs during gestation through the placenta but also to the nursing pigs from colostrum or milk Se.

Colostrum protein was approximately three fold higher than in the later milks and was not affected by dietary Se level or Se source. The form of Se in colostrum and milks for the different Se sources are unknown, but it would appear that the form when organic Se was fed was one of the seleno amino acids.
Serum Se concentrations of pigs increased by approximately two to three fold from parturition to 14 day of age. This implies that Se transferred through the placenta during gestation was less and that milk Se was efficiently transferred to the nursing pig during lactation. Young et al. (1977) reported that pig serum Se increased consistently from birth to weaning (21 day) when 1.2 ppm inorganic Se was provided to the sows.

Tissue and serum Se concentration of neonatal pigs therefore reflected the placental transfer of Se during gestation. These Se concentrations were higher as dietary Se level increased and when organic Se was fed to the gestating gilts. The increased tissue Se content in neonatal pigs from organic or inorganic Se fed to the sow has been reported previously (Mahan et al., 1977; Young et al., 1977; Nielsen et al., 1979; Chavez, 1985; Mahan and Kim, 1996).

Liver is generally considered the major labile Se storage site in the body because it declines most rapidly upon periods of Se depletion (Mahan et al., 1975; Mahan and Kim, 1996). The liver Se content in neonatal pigs was similar between 7 and 10 ppm Se from both Se sources. The reason for this plateau is unknown. However, liver Se increased as dietary Se level increased in weaning pigs even though net Se intake of sows during lactation was similar between the 7 and 10 ppm Se group of both Se sources (Table 9). This suggests that not only dietary Se but also retained Se in body tissue was mobilized and transferred to litter through the milk.

Liver Se in both the neonatal and weaning pigs were higher than any other tissues regardless of dietary Se source or level fed. Liver Se content from birth to weaning increased 2 fold when sows were fed inorganic Se, but increased by 5 fold when organic Se had been provided. This suggests that Se transfer through milk occurred more efficiently when sows were fed organic Se.

Hoof Se concentrations also increased from the neonatal to the weaning periods and was at a higher concentration compared to hair Se. This may help to explain why hoof separation in pigs occurred prior to hair loss in the inorganic Se treatment groups (Fig 9). Previous research had reported that a circular dark band in the hoofs of sows was observed during gestation when fed 16 ppm Se of sodium selenite diets (Poulsen et al., 1989). Hoof
separation occurred in the progeny of sows fed 7 to 10 ppm inorganic Se even though the Se contents were higher from pigs from sows fed organic Se was.

Hair loss in nursing pigs did not occur at < 7 ppm when organic Se was fed to the sows but did occur at 10 ppm. The hair Se concentrations were, however, subsequently higher when organic Se was fed compared with those sows fed inorganic Se. This suggests that organic Se was less toxic in weaning pigs than when inorganic Se was fed at the same dietary Se level to the sow. Because organic Se can be a component of tissue protein as selenomethionine or selenocysteine, inorganic Se cannot be directly incorporated into proteins (Ip, 1998). Consequently, excessive intermediates of inorganic Se such as selenodiglutathione (GSSeSG), glutathione selenopersulfide (GSSeH), or hydrogen selenide (H₂Se) may cause detrimental effects and (or) possess prooxidant properties in protein tissues when pigs are fed high levels of inorganic Se. Inorganic Se as sodium selenite can however, be metabolized to H₂Se via GSSeSG, and GSSeH (Ganther, 1986) in the body. These intermediates may be circulated in body fluids before they are excreted as dimethylselenide [(CH₃)₂Se] or trimethylselenonium ion [(CH₃)₃Se⁺].

Apparent digestibility of inorganic Se is higher than organic Se (Mahan and Parrett, 1996; Kim and Mahan, 1998). Consequently, more of the Se intermediates from dietary inorganic Se may accumulate in the blood before being excreted. Hoof lesions and hair loss appeared to be more severe when inorganic Se was fed even though the Se concentrations in these tissues were much lower than when organic Se was fed.

Selenium concentration in hoof and hair tissue also increased as dietary Se level increased and were highest when sows were fed organic Se. Our results suggest that the Se concentration in the hoof and hair may be used to reflect the Se status of the animal. Hunter et al. (1990) had previously demonstrated that Se content of the toenail can be a good indicator of Se status in humans. Sulfur (S) content in the hair and hoof was not affected by dietary Se level in the two Se sources. However, the total S content in the hair and hoof did not indicate any substitution of S by Se was evident in our study.

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Neither Se source or the dietary Se level affected serum GSH-Px activity in the finisher pig, sow, neonate or the nursing pig at 14 day of age. First-parity gilt GSH-Px activity appeared to plateau at .1 ppm Se when Se was provided from either sodium selenite or Se-yeast (Mahan and Kim, 1996). Thomson and Fraser (1983) suggested that serum GSH-Px activity would be the most sensitive indicator at low dietary Se levels. The results of our experiment indicate that when Se is provided at excessive dietary Se intakes, it does not appear to affect this enzyme activity.

Tissue Se contents of finisher pigs and sows at weaning increased as dietary Se level increased and were higher when the organic Se source was provided. However, organic Se deposition in muscle tissue probably represents Se that is not readily available for metabolic assimilation unless muscle catabolism takes place.

It has been suggested that the liver and kidney are more sensitive indicators of Se status of animal and previous dietary intake of the element (Jenkins and Winter, 1973). Kidney Se contents are high inorganic Se levels are fed than when organic Se was fed (Mahan and Kim. 1996; Mahan and Parrett, 1996).

Selenium concentrations in lung were lower when animals were fed organic Se, but higher when inorganic Se was provided. Diplock (1976) reported that at low Se intakes, Se is eliminated primarily in the urine, with only small amounts excreted in the bile and lungs. At high Se intakes, more Se appeared to be eliminated via the lungs as volatile dimethyl selenide (DMSe). Therefore, inorganic Se may be retained in the respiratory tissue during exhalation of DMSe. The exhaled volatile compound, DMSe, is an intermediate product of Se metabolism, which is exhaled only when its rate of formation exceeds the rate of further methylation to trimethylselenonium ion, a urinary Se metabolite (Glover et al., 1979).

Serum Se in all pigs was similar at 49 day of age when pigs were fed postweaning diets which contained .3 ppm Se (inorganic Se). Although this does not reflect the tissue Se status of the pigs, it does imply that mobilization of labile Se stores was limited.
3.6 Implications

Toxic levels of Se in reproducing sow was examined when high Se diets were fed from 25 kg through a complete reproductive cycle. Above 7 ppm inorganic Se, Se toxicity occurred in the progeny of sows fed inorganic Se, whereas 10 ppm organic Se reduced reproductive performance of sows. GSH-Px activity of sows and pigs were not affected by dietary Se level or source. Selenium transfer through placenta or milk was higher when organic was provided during gestation and lactation. Tissue and serum Se concentrations of sows and neonatal pigs were higher when sows were fed organic Se. Hair loss (alopecia) and hoof separation occurred more frequently and at lower dietary Se levels in nursing pigs when inorganic Se was fed to the sow.
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Grower&lt;sup&gt;ab&lt;/sup&gt;</th>
<th>Finisher&lt;sup&gt;ac&lt;/sup&gt;</th>
<th>Gestation&lt;sup&gt;ad&lt;/sup&gt;</th>
<th>Lactation&lt;sup&gt;ae&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>79.30</td>
<td>84.45</td>
<td>76.26</td>
<td>71.92</td>
</tr>
<tr>
<td>Soybean meal, 44% CP</td>
<td>17.50</td>
<td>13.00</td>
<td>19.83</td>
<td>19.95</td>
</tr>
<tr>
<td>Fat&lt;sup&gt;f&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.00</td>
</tr>
<tr>
<td>Salt&lt;sup&gt;g&lt;/sup&gt;</td>
<td>.50</td>
<td>.35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-Lysine • HCl</td>
<td>.20</td>
<td>.20</td>
<td>-</td>
<td>0.20</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.30</td>
<td>1.00</td>
<td>1.93</td>
<td>1.99</td>
</tr>
<tr>
<td>Limestone</td>
<td>.85</td>
<td>.70</td>
<td>1.07</td>
<td>1.04</td>
</tr>
<tr>
<td>Trace mineral&lt;sup&gt;i,j&lt;/sup&gt;</td>
<td>.05</td>
<td>.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TM salt&lt;sup&gt;h&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Vitamin mix&lt;sup&gt;k,l&lt;/sup&gt;</td>
<td>0.20</td>
<td>0.20</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Antibiotic&lt;sup&gt;k,l&lt;/sup&gt;</td>
<td>.10</td>
<td>.05</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sodium selenite or the Se-Yeast was premixed in ground corn (200 mg/kg) and added to the diet at the appropriate treatment levels.

<sup>b</sup> Formulated to .80% lysine, .65% Ca, and .55% P.

<sup>c</sup> Formulated to .75% lysine, .55% Ca, and .50% P.

<sup>d</sup> Formulated to .75% lysine, .90% Ca, and .70% P.

<sup>e</sup> Formulated to .90% lysine, .90% Ca, and .70% P.

<sup>f</sup> Animal vegetable blend

<sup>g</sup> Supplied per kilogram diet: 10 mg of Cu (copper oxide); 100 mg of Fe (ferrous sulfate); .2 mg of I (calcium iodate); 40 mg of Mn (manganese oxide); 120 mg of Zn (zinc oxide).

<sup>h</sup> Supplied per kilogram diet: 8 mg of Cu (copper oxide); 120 mg of Fe (ferrous sulfate); .2 mg of I (calcium iodate); 15 mg of Mn (manganese oxide); 120 mg of Zn (zinc oxide); and 4.22 g of NaCl.

<sup>i</sup> Supplied per kilogram in the grower-finisher diet: 1,750 IU of vitamin A; 200 IU of vit D<sub>3</sub>; 11 IU of vitamin E; .5 mg of vitamin K; 3.0 mg of riboflavin; 10 mg of pantothenic acid; 13 mg of niacin; .3 mg of folacin; .05 mg of biotin; 15 ug of vitamin B<sub>12</sub>; .4 g of choline and 66 mg of BHT.

<sup>j</sup> Supplied per kilogram in the gestation-lactation diet: 4,000 IU of vitamin A (acetate); 220 IU of vit D<sub>3</sub>; 40 IU of vitamin E (acetate); .5 mg of vitamin K (menadione); 15 mg of d-pantothenic acid; 4 mg of riboflavin; 12 mg of niacin; 1 mg of folacin; .2 mg of d-biotin; 20 ug of vitamin B<sub>12</sub>; 1.25 g of choline; and 66 mg of butylated hydroxytoluene (BHT) as an antioxidant.

<sup>k</sup> Tylosin was added at 10 mg per kilogram diet for the grower, finisher, and gestation diets.

<sup>l</sup> Bacitracin methylene disalicylate (BMD) was added at 330 mg per kilogram diet during lactation.

Table 8. Percentage composition of experimental diets (as fed)
<table>
<thead>
<tr>
<th>Se Source</th>
<th>Inorganic</th>
<th>Organic</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se Level, ppm</td>
<td>.3</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Weight, kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final</td>
<td>106.70</td>
<td>101.80</td>
<td>99.50</td>
</tr>
<tr>
<td>Daily gain, kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 96 d</td>
<td>.86</td>
<td>.82</td>
<td>.77</td>
</tr>
<tr>
<td>Daily feed intake, kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 96 d</td>
<td>2.51</td>
<td>2.32</td>
<td>2.21</td>
</tr>
<tr>
<td>Gain : Feed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 96 d</td>
<td>.342</td>
<td>.354</td>
<td>.349</td>
</tr>
<tr>
<td>Serum Se, ppm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>.119</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6 wk</td>
<td>.179</td>
<td>.575</td>
<td>.837</td>
</tr>
<tr>
<td>14 wk</td>
<td>.149</td>
<td>.562</td>
<td>.670</td>
</tr>
<tr>
<td>Liver Se, ppm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>.506</td>
<td>2.811</td>
<td>2.952</td>
</tr>
</tbody>
</table>

<sup>a</sup> Dietary Se source response (P < .05).
<sup>b</sup> Dietary Se source response (P < .01).
<sup>c</sup> Dietary Se level linear response (P < .05).
<sup>d</sup> Dietary Se level linear response (P < .01).
<sup>e</sup> Dietary Se level x Se source interaction response (P < .01).

**Table 9.** Treatment effects of dietary selenium source and selenium level on growth performance, serum and liver Se of grower - finisher pigs
<table>
<thead>
<tr>
<th>Se source :</th>
<th>Inorganic</th>
<th>Organic</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se Level, ppm :</td>
<td>.3</td>
<td>.3</td>
<td>7&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. sow bred</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>No. farrowed</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Sow feed intake, kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>67.6</td>
<td>47.0</td>
<td>67.4</td>
</tr>
<tr>
<td>ADMI</td>
<td>4.83</td>
<td>3.26</td>
<td>4.83</td>
</tr>
<tr>
<td>Weight, kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeding</td>
<td>145</td>
<td>145</td>
<td>139</td>
</tr>
<tr>
<td>110 d</td>
<td>196</td>
<td>199</td>
<td>196</td>
</tr>
<tr>
<td>Gain (0 – 109 d)</td>
<td>51</td>
<td>54</td>
<td>58</td>
</tr>
<tr>
<td>Farrowing</td>
<td>184</td>
<td>177</td>
<td>178</td>
</tr>
<tr>
<td>Wean (14 d)</td>
<td>187</td>
<td>181</td>
<td>183</td>
</tr>
<tr>
<td>Gain (0 -14 d)</td>
<td>3</td>
<td>-1</td>
<td>6</td>
</tr>
<tr>
<td>Backfat, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeding</td>
<td>19.3</td>
<td>15.8</td>
<td>13.7</td>
</tr>
<tr>
<td>110 d</td>
<td>21.7</td>
<td>17.3</td>
<td>15.7</td>
</tr>
<tr>
<td>Farrowing</td>
<td>21.0</td>
<td>18.3</td>
<td>16.3</td>
</tr>
<tr>
<td>Wean (14 d)</td>
<td>19.7</td>
<td>17.3</td>
<td>15.7</td>
</tr>
<tr>
<td>Parturition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. pigs</td>
<td>9.67</td>
<td>10.75</td>
<td>10.33</td>
</tr>
<tr>
<td>No. stillborn pigs</td>
<td>.33</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>No. pigs, live</td>
<td>9.34</td>
<td>9.75</td>
<td>9.33</td>
</tr>
<tr>
<td>Litter wt, kg</td>
<td>14.00</td>
<td>14.74</td>
<td>14.42</td>
</tr>
<tr>
<td>Pig wt, kg</td>
<td>1.48</td>
<td>1.41</td>
<td>1.40</td>
</tr>
<tr>
<td>14 day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. pigs</td>
<td>8.33</td>
<td>9.67</td>
<td>8.00</td>
</tr>
<tr>
<td>Litter wt, kg</td>
<td>32.99</td>
<td>34.26</td>
<td>33.14</td>
</tr>
<tr>
<td>Pig wt, kg</td>
<td>4.02</td>
<td>3.61</td>
<td>4.07</td>
</tr>
<tr>
<td>Litter gain (0 -14 d), kg</td>
<td>18.99</td>
<td>19.03</td>
<td>18.71</td>
</tr>
<tr>
<td>Pig gain (0 - 14 d), kg</td>
<td>2.45</td>
<td>2.23</td>
<td>2.67</td>
</tr>
</tbody>
</table>

<sup>a</sup> Severe alopecia occurred in the nursing pigs by 7 days postpartum.
<sup>b</sup> Dietary Se source response (P < .05).
<sup>c</sup> Dietary Se level linear response (P < .05).
<sup>d</sup> Dietary Se level linear response (P < .01).
<sup>e</sup> Dietary Se level x Se source response (P < .05).
<sup>f</sup> Dietary Se level x Se source response (P < .01).

Table 10. Treatment effects of dietary selenium source and selenium level on reproducing sow performance responses
<table>
<thead>
<tr>
<th>Se source :</th>
<th>Inorganic</th>
<th>Organic</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se Level, ppm :</td>
<td>.3</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>No. of sows</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Sow serum Se, ppm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeding</td>
<td>.155</td>
<td>.428</td>
<td>.767</td>
</tr>
<tr>
<td>110 d</td>
<td>.151</td>
<td>.289</td>
<td>.581</td>
</tr>
<tr>
<td>Wean (14 d)</td>
<td>.193</td>
<td>.382</td>
<td>.649</td>
</tr>
<tr>
<td>Sow blood profile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>110 d</td>
<td>13.39</td>
<td>11.75</td>
<td>11.85</td>
</tr>
<tr>
<td>Wean (14 d)</td>
<td>12.83</td>
<td>10.70</td>
<td>12.36</td>
</tr>
<tr>
<td>Packed cell volume, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>110 d</td>
<td>30.67</td>
<td>34.65</td>
<td>36.87</td>
</tr>
<tr>
<td>Wean (14 d)</td>
<td>35.03</td>
<td>31.80</td>
<td>33.03</td>
</tr>
<tr>
<td>Red blood cells No., x10^12/L</td>
<td>5.560</td>
<td>5.515</td>
<td>-</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>.30</td>
<td>.50</td>
<td>-</td>
</tr>
<tr>
<td>No. x 10^9/L</td>
<td>16.45</td>
<td>27.55</td>
<td>-</td>
</tr>
<tr>
<td>Sow GSH-Px, unit/mL a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 d</td>
<td>.530</td>
<td>.608</td>
<td>.602</td>
</tr>
<tr>
<td>110 d</td>
<td>.453</td>
<td>.543</td>
<td>.453</td>
</tr>
<tr>
<td>Wean (14 d)</td>
<td>.503</td>
<td>.575</td>
<td>.685</td>
</tr>
</tbody>
</table>

a One unit of activity equals 1 umol NADPH oxidized per minute/mL serum.
^b Dietary Se source response (P < .01).
^c Dietary Se level linear response (P < .05).
^d Dietary Se level linear response (P < .01).
^e Dietary Se level x Se source response (P < .05).
^f Dietary Se level x Se source response (P < .01).

Table 11. Treatment effects of dietary selenium source and selenium level on sow blood measurements
<table>
<thead>
<tr>
<th>Se Source :</th>
<th>Inorganic</th>
<th></th>
<th>Organic</th>
<th></th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se Level :</td>
<td>.3</td>
<td>.3</td>
<td>7</td>
<td>10</td>
<td>.3</td>
</tr>
<tr>
<td>No. of sows</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Milk Se, ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 d</td>
<td>.235</td>
<td>.445</td>
<td>.850</td>
<td>1.211</td>
<td>.332</td>
</tr>
<tr>
<td>1 d</td>
<td>.189</td>
<td>.241</td>
<td>.445</td>
<td>1.635</td>
<td>.218</td>
</tr>
<tr>
<td>2 d</td>
<td>.117</td>
<td>.180</td>
<td>.361</td>
<td>1.599</td>
<td>.139</td>
</tr>
<tr>
<td>3 d</td>
<td>.057</td>
<td>.148</td>
<td>.306</td>
<td>1.070</td>
<td>.103</td>
</tr>
<tr>
<td>4 d</td>
<td>.051</td>
<td>.138</td>
<td>.299</td>
<td>.818</td>
<td>.096</td>
</tr>
<tr>
<td>5 d</td>
<td>.056</td>
<td>.159</td>
<td>.293</td>
<td>.922</td>
<td>.090</td>
</tr>
<tr>
<td>6 d</td>
<td>.055</td>
<td>.149</td>
<td>.292</td>
<td>.981</td>
<td>.094</td>
</tr>
<tr>
<td>7 d</td>
<td>.045</td>
<td>.111</td>
<td>.357</td>
<td>.797</td>
<td>.086</td>
</tr>
<tr>
<td>10 d</td>
<td>.055</td>
<td>.159</td>
<td>.345</td>
<td>1.264</td>
<td>.090</td>
</tr>
<tr>
<td>14 d</td>
<td>.043</td>
<td>.166</td>
<td>.179</td>
<td>.761</td>
<td>.089</td>
</tr>
<tr>
<td>Milk protein, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 d</td>
<td>18.27</td>
<td>17.39</td>
<td>17.73</td>
<td>18.09</td>
<td>18.40</td>
</tr>
<tr>
<td>2 d</td>
<td>7.83</td>
<td>7.85</td>
<td>7.83</td>
<td>8.02</td>
<td>9.03</td>
</tr>
<tr>
<td>3 d</td>
<td>6.84</td>
<td>6.45</td>
<td>6.61</td>
<td>6.25</td>
<td>6.79</td>
</tr>
<tr>
<td>4 d</td>
<td>6.55</td>
<td>5.49</td>
<td>6.16</td>
<td>6.53</td>
<td>6.29</td>
</tr>
<tr>
<td>5 d</td>
<td>6.69</td>
<td>5.76</td>
<td>6.46</td>
<td>5.64</td>
<td>5.77</td>
</tr>
<tr>
<td>6 d</td>
<td>6.61</td>
<td>5.30</td>
<td>7.07</td>
<td>6.01</td>
<td>5.66</td>
</tr>
<tr>
<td>7 d</td>
<td>5.79</td>
<td>5.26</td>
<td>5.95</td>
<td>5.40</td>
<td>5.75</td>
</tr>
<tr>
<td>10 d</td>
<td>6.62</td>
<td>4.93</td>
<td>5.49</td>
<td>5.44</td>
<td>5.53</td>
</tr>
<tr>
<td>14 d</td>
<td>5.43</td>
<td>4.83</td>
<td>5.50</td>
<td>5.46</td>
<td>5.33</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Dietary Se source response (P < .05).
\textsuperscript{b} Dietary Se source response (P < .01).
\textsuperscript{c} Dietary Se level linear response (P < .05).
\textsuperscript{d} Dietary Se level linear response (P < .01).
\textsuperscript{e} Dietary Se level x Se source response (P < .05).
\textsuperscript{f} Dietary Se level x Se source response (P < .01).

Table 12. Treatment effects of dietary selenium source and selenium level on sow milk Se and milk protein

96
<table>
<thead>
<tr>
<th>Se Source</th>
<th>Inorganic</th>
<th>Organic</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3  3  7  10</td>
<td>3  3  7  10</td>
<td></td>
</tr>
<tr>
<td>Se Level, ppm:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loin</td>
<td>.147  .166  .239  .226</td>
<td>.277  1.694  4.142  5.326</td>
<td>.14&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver</td>
<td>.578  2.114  3.604  3.432</td>
<td>.753  2.768  7.158  8.932</td>
<td>.65&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lung</td>
<td>.211  .386  .789  .904</td>
<td>.302  1.362  2.904  4.018</td>
<td>.12&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pancreas</td>
<td>.389  .545  .658  .903</td>
<td>.547  2.441  5.409  6.504</td>
<td>.38&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spleen</td>
<td>.344  .655  1.617</td>
<td>.415  1.586  4.217  5.200</td>
<td>.27&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.136  2.556  3.987  4.422</td>
<td>2.490  4.990  8.684  9.287</td>
<td>.79&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hoof</td>
<td>.273  .502  1.423  1.995</td>
<td>.838  3.610  16.167  12.272</td>
<td>.8&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hair</td>
<td>.487  1.383  2.149  2.832</td>
<td>.860  5.121  7.959  10.532</td>
<td>.39&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- Dietary Se source response (P < .05).
- Dietary Se level linear response (P < .01).
- Dietary Se level x Se source response (P < .05).
- Dietary Se level x Se source response (P < .01).

Table 13. Treatment effects of dietary selenium source and selenium level on tissue Se of sow
<table>
<thead>
<tr>
<th>Se Source : Inorganic</th>
<th>Organic</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se Level, ppm :</td>
<td>3 3 7 10</td>
<td>3 3 7 10</td>
</tr>
<tr>
<td>No. of pig</td>
<td>3 4 3 3</td>
<td>3 2 3 3</td>
</tr>
<tr>
<td>Pig serum Se, ug/mL</td>
<td></td>
<td>.042 .097 .141 .155</td>
</tr>
<tr>
<td>0 d</td>
<td>.062 .121 .237 .300</td>
<td>.093 .366 1.041 1.430</td>
</tr>
<tr>
<td>7 d</td>
<td>.074 .136 .220 .306</td>
<td>.092 .412 1.143 1.418</td>
</tr>
<tr>
<td>14 d (weaning)</td>
<td>.120 .130 .134 -</td>
<td>.126 - .220 .203 .01</td>
</tr>
<tr>
<td>49 d</td>
<td></td>
<td>.073 .135 .135 .194</td>
</tr>
<tr>
<td>Hemoglobin, %</td>
<td>0 d</td>
<td>11.8 9.3 12.0 10.4</td>
</tr>
<tr>
<td>7 d</td>
<td>11.8 9.2 10.4 10.8</td>
<td>11.0 10.8 10.8 10.8</td>
</tr>
<tr>
<td>14 d</td>
<td>13.1 11.3 11.8 12.1</td>
<td>11.6 13.5 12.9 11.2</td>
</tr>
<tr>
<td>Blood packed cell volume, %</td>
<td>0 d</td>
<td>40.2 39.9 41.2 41.6</td>
</tr>
<tr>
<td>7 d</td>
<td>37.5 32.4 34.9 35.1</td>
<td>35.7 36.1 34.2 36.7</td>
</tr>
<tr>
<td>14 d</td>
<td>42.0 34.5 37.4 39.2</td>
<td>38.7 40.1 39.0 36.8</td>
</tr>
<tr>
<td>Pig GSH-Px, unit/mL</td>
<td></td>
<td>.111 .174 .193 .225</td>
</tr>
<tr>
<td>a</td>
<td></td>
<td>.235 .360 .344 .211</td>
</tr>
<tr>
<td>b</td>
<td></td>
<td>.270 .318 .318 -</td>
</tr>
<tr>
<td>Sulfur content (14 d), %</td>
<td>Hair</td>
<td>4.093 - - 4.020 4.100 - - 4.130 4.130</td>
</tr>
<tr>
<td>Hoof</td>
<td>1.890 - - 1.980 1.920 - - 1.570 1.570</td>
<td></td>
</tr>
</tbody>
</table>

\a One unit of activity equals 1 umol NADPH oxidized per minute/mL serum.
\b Dietary Se source response (P < .01).
\c Dietary Se level linear response (P < .05).
\d Dietary Se level linear response (P < .01).
\e Dietary Se level x Se source response (P < .05).
\f Dietary Se level x Se source response (P < .01).

Table 14. Treatment effects of dietary selenium source and selenium level on pig blood measurements
<table>
<thead>
<tr>
<th>Pig tissue Se, ppm</th>
<th>Neonate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Weaning, 14 d</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pig</td>
<td>Neutropenic pigs</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>.010</td>
<td>.049</td>
<td>.115</td>
</tr>
<tr>
<td>Pancreas</td>
<td>.060</td>
<td>.150</td>
<td>.316</td>
</tr>
<tr>
<td>Kidney</td>
<td>.390</td>
<td>.592</td>
<td>.893</td>
</tr>
<tr>
<td>Hoof</td>
<td>.062</td>
<td>.387</td>
<td>1.136</td>
</tr>
<tr>
<td>Hair</td>
<td>.484</td>
<td>1.115</td>
<td>2.319</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pig tissue Se, ppm</th>
<th>Neutropenic pigs</th>
<th>Weaning, 14 d</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pig</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loin</td>
<td>.104</td>
<td>.109</td>
<td>.132</td>
</tr>
<tr>
<td>Liver</td>
<td>.489</td>
<td>3.244</td>
<td>11.899</td>
</tr>
<tr>
<td>Lung</td>
<td>.126</td>
<td>.212</td>
<td>.240</td>
</tr>
<tr>
<td>Pancreas</td>
<td>.174</td>
<td>.233</td>
<td>.302</td>
</tr>
<tr>
<td>Kidney</td>
<td>.682</td>
<td>.709</td>
<td>.879</td>
</tr>
<tr>
<td>Hoof</td>
<td>.309</td>
<td>.510</td>
<td>1.063</td>
</tr>
<tr>
<td>Hair</td>
<td>.323</td>
<td>.732</td>
<td>1.520</td>
</tr>
</tbody>
</table>

<sup>a</sup> Neonatal pigs were killed prior to the consumption of colostrum.
<sup>b</sup> Dietary Se source response (P < .01).
<sup>c</sup> Dietary Se level linear response (P < .01).
<sup>d</sup> Dietary Se level x Se source interaction (P < .01).

Table 15. Treatment effects of dietary selenium source and selenium level on tissue Se of neonatal and weaning pig.

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Figure 8. Hair loss (alopecia) in nursing pig by 14 day of age when 7 ppm inorganic Se was provided to the sow.

Figure 9. Hoof separation and sloughing of the nursing pig by 14 day of age when sow was fed 7 ppm inorganic Se.
CHAPTER 4

Hair Measurements as an Indicator of Selenium Status of Pigs

4.1 Abstract

Two experiments evaluated the effects of dietary Se levels, Se source and hair color on the resultant hair Se concentration, amino acids profile and hair diameter of grower-finisher pigs and adult gilts. The first experiment was conducted in a 2 x 6 factorial arrangement in a split-plot design. An inorganic and organic Se source were added at .3, 1, 3, 5, 7 and 10 ppm Se. A total of 48 crossbred pigs were fed their treatment diets from 25 kg to 105 kg in two replicates. Hair samples were collected from the dorsal-midline at 4, 6, 10, 12, and 14 wk after feeding the treatment diets and analyzed for Se and amino acids. Hair Se concentration increased as dietary Se level increased ($P < .01$) and was higher when pigs were fed organic Se ($P < .05$). Selenium concentration in red colored hair was higher than white color hair ($P < .01$). The coefficients of correlation between dietary Se level and hair Se concentration averaged $r = .89$ and was higher when organic Se was fed. The amino acid compositions were similar between white and red color hair, and for Se sources and levels.

The second experiment was conducted with three different purebred gilts, at 9 mo. of age. Gilts had been fed diets that contained .3 ppm inorganic Se. Hair samples were collected from different locations (shoulder, back, rump, front-leg, belly, hind-leg) to determine if hair Se content
and hair diameter differed by location. Hair Se concentration was the highest in red and white haired pigs, lowest in the black haired gilts ($P < .01$). The diameter of red colored hair tended to be thicker than white or black hair ($P < .22$). Hair Se concentration in the lower body extremities appeared to be higher than hair from the upper parts of the body. Hair diameter was not affected by location. These results suggest that hair Se can be a good indicator of Se status when pigs were fed the toxic levels of Se. Se concentration in hair was varied from hair color although pigs were fed the same diets. Selenium requirements may differ by breed or hair color.

Key Words: Selenium, Hair, Pigs

4.2 Introduction

Hair growth is affected by the amount of nutrient content in the circulating extracellular fluid during hair formation (Hopps, 1977). Consequently, it has been suggested that the analysis of hair might provide information about the mineral status of domestic animals (Reinhold et al., 1968; Sims, 1968; Hall et al., 1971). Wysocki and Klett (1971) and Hopps (1977) proposed that sweat secreted by the sebaceous glands may also contribute minerals to the hair and that the fatty secretions of apocrine glands may provide a physical or chemical means by which exogenous minerals may absorb onto hair. The mineral content of hair can be affected by season, breed, hair color sire, age and body location (Combs et al., 1982).

Selenium concentration in the hair seems to be correlated with dietary Se intake. Perry et al. (1976) reported that when sodium selenite was fed to beef cattle the correlation between dietary Se level and hair Se concentration was .90. Gehringer et al. (1984b) reported that hair Se concentrations in pigs increased quadratically as the level of supplemental Se increased. Wahlstrom et al. (1984) demonstrated that hair Se content in swine can be affected by dietary Se level but hair color seemed to have an important relationship to its Se concentration.
Stowe and Miller (1985) demonstrated that hyper-selenemic pigs had a higher growth rate and a higher serum Se concentration than did the hypo-selenemic pigs. An increase in serum Se resulted when dietary Se was supplemented was greater among the hypo-seleemic pigs. Serum GSH-Px activity for the hyperselenemic pigs was higher in hyposelenemic pigs. These results suggest that pigs have different genetic responses to dietary Se and may have different Se requirements.

The objective of this experiment was to evaluate the effect of organic or inorganic Se at various levels on resulting hair Se concentration and the influence of hair color on hair Se content, hair amino acids profile, and hair diameter in swine.

4.3 Material and Methods

Grower-Finisher Pigs: A previous experiment had been conducted to evaluate the effects of various dietary levels of organic and inorganic Se sources on the selenosis condition in grower-finisher pigs (Chapter 2). In that study it was observed that pigs within each treatment pen particularly those fed the higher Se diets varied widely in their body weights and that these differences seemed to be by hair color. We speculated that if Se was retained more effectively in different hair colors, then the available Se to body tissue could affect the expression of the selerosis condition.

Hair samples were subsequently collected from the different colored haired pigs within each treatment pen at 4, 6, 10, 12, and 14 wk from the dorsal-midline of pigs after treatment diets were fed. The hair was brushed before clipping to remove adhered foreign material. Hair samples were only obtained from fully developed hair. Selenium analysis was subsequently conducted on all hair samples with amino acids analyzed from the 6 wk samples.

Adult Gilt: Three different breeds (Yorkshire, Duroc and Hampshire) of gilts at approximately 9 mo. of age and which had been fed diets which contained .3 ppm inorganic Se were used to
evaluate their hair Se contents and diameters at various body locations. Samples were collected in September 1998 from a private farm (Isler Genetics, Prospect OH). Hair samples were collected from the shoulder, back, rump, front-leg, belly, and hind-leg areas from gilts.

Previous research had demonstrated that the mineral concentration in hair varied with season in humans (Strain et al., 1966), cattle (Miller, 1965; O'Mary et al., 1969), and ponies (Wysocki and Klett, 1971).

**Analytical Methods:** Hair samples were washed three times with 60 °C distilled water, dried and analyzed for Se, diameter measured, and amino acid content. Hair Se analyses were conducted by the fluorometric method (AOAC, 1996) after wet ashing in perchloric acid and nitric acid. Hair diameter was measured by placing each hair strand under microscope (Olympus DP10, NY) measuring at the midpoint and at the midpoint toward each end and using the average value for each strand.

Amino acids were analyzed by gas-liquid chromatography (Picotag, Waters MT) as outlined by Kaiser et al. (1974). In this procedure the use of ultrasonication under vacuum to remove dissolved air before acid hydrolysis (6 N HCl) prevented the oxidation of the sulfur amino acids. Tryptophan was analyzed separately after hydrolysis in an alkaline medium.

Statistical analyses of the grower-finisher pig were conducted using the GLM procedure of SAS (1985) using a split-plot design. Gilt hair was analyzed using as a CRD design with breed and body location as the treatment variables. The individual animal was considered the experiment unit.

### 4.4 Results

Hair Se concentration increased as dietary Se level increased at each measurement period (P < .01) and was higher when organic Se was provided, after the 12 wk period (P < .05; Table 16). The hair Se concentration was higher in red colored rather than white colored hair (P < .01) as
dietary Se level increased \((P < .01)\). When pigs were fed diets that contained .3 ppm Se, the resulting hair Se concentration decreased as pigs grew but increased during the latter part of the finisher period. Hair Se concentrations increased as dietary Se level increased and was higher in red colored hair, resulting in an interaction response \((P < .01; \text{Table 17})\). Hair Se concentration was higher in red hair colored pigs within 4 wk but the magnitude of the difference between the two colors increased with age, resulting in an age x hair color interaction response \((P < .01; \text{Table 18})\). Hair Se concentration increased when pigs were fed organic Se and dietary Se level particularly in red colored hair. This resulted in a three way Se level x Se source x hair color interaction response \((P < .05; \text{Table 19})\).

Body size of white pigs was consistently smaller than red or black colored pigs when pigs were fed high dietary levels of Se. Selenosis symptoms such as hair loss and hoof separation occurred more frequently in white colored hair pigs within each of the treatment diets (Fig. 5 and 9). Unfortunately there was not an equal distribution of hair color between pens and resulting growth responses to the dietary variable could not be partitioned.

Coefficients of correlation \((r)\) between dietary Se level and hair Se concentration was somewhat higher in red haired than white haired pigs in both Se sources (Table 20). The correlation was consistently higher when pigs were fed organic Se after 10 wk of the trial.

Hair amino acid compositions were not affected by dietary Se level or Se source (Table 21). The essential amino acid, arginine, had a higher Se content in red than white haired pigs when diets contained inorganic Se, Se content was higher in white haired pigs than when organic Se was provided. This resulted in a Se source x hair color interaction response \((P < .05; \text{Table 22})\). The reason for this response is unknown. Methionine concentration was not affected by feeding organic Se which had a high selenomethionine content. The organic Se provided to these pigs contained various seleno amino acids and seleno analogs but the principle one was selenomethionine (Kelly and Power, 1995). The nonessential amino acids glutamic acid and hydroxyproline contents also showed differing responses which resulted in Se source x hair color interaction response \((P < .05)\). Cysteine content was the highest amino acid in swine hair
regardless of dietary Se, level, source or hair color but was not affected by any of the treatment variables (Table 22).

In experiment 2 using adult gilts, hair Se concentration was highest in the red and white haired \( (P < .01) \) breed lowest in the black hair breed (Table 23). The diameter of red hair was thicker than the white or black hair (Table 23). Hair Se in the shoulder had the lowest Se concentration and had the highest concentration in hind-leg. In all cases hair Se appeared to be higher in the posterior and in the lower portions of the body.

There were morphological differences in hair strands from the three breeds. White hair strands had split into several pieces at the end (Fig. 10a), while black and red hair remained intact and were thicker (Fig. 10b).

4.5 Discussion

Hair consists of an inner medulla, an outer cortex, and a thin cuticle covering (Frandsen and Spurgeon, 1992). The medulla may contain a pigment, which has little effect on hair color, but the space between the medullary cells is believed to yield a white or silver color to the hair if the cortex lacks pigment. Consequently, the major portion of hair is from the cortex, which consists of several layers of cornified cells. The amount and type of melanin in the cortical cells determines whether hair color within the cuticle is a single layer of thin, clear cells which cover the surface of the cortex (Frandsen and Spurgeon, 1992).

Hair regrowth may be altered slightly by physical responses of the animal. Plucking hair effectively stimulates hair growth, but the cutting of hair when there is no damage to the follicle has little effect on the growth of hair (Hopps, 1977). Wahstrom et al. (1984) had previously demonstrated that hair Se concentration was higher in hair regrowth than when collected from an unclipped area.
This experiment demonstrated that hair Se concentration increased as dietary Se level increased and was higher when it was a red color. Pigs fed organic Se had hair of a higher Se concentration than those fed inorganic Se. Hidiroglou et al. (1965) had previously demonstrated that cows with hair Se concentrations between .06 and .23 ppm produced calves having white muscle disease, but no deficiency lesions were found in calves from cows with hair Se content greater > .25 ppm. Olson (1969) reported that a continuous intake of 5 ppm Se by cattle resulted in selenosis and that a concentration of 5 to 10 ppm Se in the hair of these cattle indicated Se toxicity. In our previous experiment (Chapter 3) there were body areas which contained both white and/or red colored hair and animals that had a high proportion of each color. When sows were fed 7 ppm inorganic Se, hair Se concentration of neonatal pig was 2.112 and 4.435 ppm in white and red colored hair, respectively. Because selenosis symptoms occurred at a lower dietary Se level when pigs were fed inorganic Se, those pigs with a white hair color demonstrated more severe selenosis symptoms at a lower dietary Se concentration. Selenosis symptoms, hair loss and hoof separation occurred at 1.6 ppm Se in the hair of nursing pigs when inorganic Se was fed to their lactationary dams these conditions did not occur until 10 ppm when organic Se was fed (Chapter 3). In addition, hair loss was more severe in the white colored hair areas of the body although Se concentration was higher in red hair (Chapter 3).

Wahlstrom et al. (1984) had previously demonstrated, however, that Se concentration in black and white haired pigs contained more Se than in the red colored hair.

Maugh (1978) demonstrated that hair Se concentration is approximately 10 fold higher than the Se content in the serum or urine. Our previous experiment also showed that hair Se concentration was 5 to 10 fold higher than the serum Se concentration in both the nursing pig and sow (Chapter 3).

The sulfur-containing amino acid cysteine had the highest amino acid concentration in swine hair and was not affected by dietary Se level, Se source or hair color. Hair is high in the sulfur-containing amino acids (i.e. cysteine) that, presumably provides ligands for stable hair-mineral bonds (Hinners et al., 1974; Hopps, 1977). Mahan and Shields, (1998) demonstrated that
cysteine had the highest amino acid concentration in swine hair. Cysteine is easily destroyed when processed into hair meal by heating and pressure. Consequently, it has been reported that the cysteine concentration in commercial hair meal was low (Wang and Parsons, 1997).

Methionine concentration was not affected by Se source in our study or that of Olson and Palmer (1976).

Hall et al. (1971) demonstrated that hair grew more rapidly during colder weather in order to provide surface insulation protection for the animal. Consequently, seasonal affects on hair composition can influence stage of growth, changes caused by perspiration, surface contamination, and dietary mineral composition.

The morphological differences between white and black colored hair that we found in the adult gilts suggests that white hair appeared to have a weaker structure and was more fragile than dark colored hair. O'Mary et al. (1969) demonstrated that the ash percentage and Ca, P, Mg, Mn, Fe and K contents were higher in red than white haired cattle. Combs et al. (1982) also reported that pigmented hair was higher in Ca, Mg, K, and Na than white hair, but the other trace mineral concentrations were similar in the hairs of different colors.

White hair located at the lower body areas (front-leg, hind-leg and belly) was tarnished, damaged and contaminated by urine and/or feces. Consequently, hair Se concentrations were higher in the lower body areas than the upper areas. Flynn (1977) suggested that elements can be adsorbed to the hair surface, whether from endogenous secretions or from exogenous contamination. Consequently, the hair mineral analyses may also have contained adsorbed minerals (Hambridge et al., 1972). Hunter et al. (1990) reported that the Se in toenails can be a good indicator of Se status in humans because this tissue was less prone to superficial contamination than hair.

Combs, (1987) demonstrated that hair growth in many animals progressed posteriorly and dorsally as seasonal moults occur. Although the location of hair can affect hair mineral concentration, hair color seems to more important than its location on the body in regards to mineral composition (O'Mary et al., 1969).
4.6 Implications

Hair Se concentration can be a good indicator of the pigs Se status and is not affected by Se source. Colored haired pigs were more resistant to selenosis compared with white haired pigs because their hair was an avenue of Se excretion. Amino acid composition of pig hair was similar for all hair colors evaluated. Red color hair contained more Se and was thicker than white color and hair Se content. Consequently, the pigs Se requirement may be influenced by hair Se retention. Hair Se content and diameter were affected by hair location of the body.
### Table 16. Main effects of dietary selenium level, hair color, and age on hair selenium concentration of grower-finisher pigs

<table>
<thead>
<tr>
<th>Hair Color</th>
<th>Se Level</th>
<th>Age, Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>Red</td>
<td>SEM</td>
</tr>
<tr>
<td>No. of samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair Se, ppm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Dietary Se source response (P < .01).

<sup>b</sup> Hair Color response (P < .01).

<sup>c</sup> Age response (P < .01).

### Table 17. Treatment effects of dietary selenium level and hair color on hair selenium concentration of grower-finisher pigs

<table>
<thead>
<tr>
<th>Hair Color</th>
<th>White</th>
<th>Red</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se Level :</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Se Level</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>No. of samples</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

<sup>a</sup> Dietary selenium level x hair color interaction response (P < .01).
<table>
<thead>
<tr>
<th>Hair Color</th>
<th>White</th>
<th>Red</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, Week</td>
<td>4  6  10  12 14</td>
<td>4  6  10  12 14</td>
</tr>
<tr>
<td>No. of samples</td>
<td>24  24  24  24  24</td>
<td>19  22  20  22  22</td>
</tr>
<tr>
<td>Hair Se, ppm</td>
<td>3.031  3.500  4.264  4.541  5.039</td>
<td>3.585  5.463  7.155  7.084  9.338</td>
</tr>
</tbody>
</table>

* Hair color x age interaction response (P < .01).

Table 18. Treatment effects of hair color and age on hair selenium concentration of grower-finisher pigs

<table>
<thead>
<tr>
<th>Se Source</th>
<th>Inorganic</th>
<th>Organic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se Source</td>
<td>Hair Color</td>
<td>White</td>
</tr>
<tr>
<td>Se Level</td>
<td>3  1  3  5  7  10</td>
<td>.3  1  3  5  7  10</td>
</tr>
<tr>
<td>No. of samples</td>
<td>10  10  10  10  10  10</td>
<td>9  9  7  10  10  5</td>
</tr>
</tbody>
</table>

* Dietary selenium source x hair color x selenium level interaction response (P < .05).

Table 19. Treatment effects of dietary selenium source, hair color, and selenium level on hair selenium concentration of grower-finisher pigs
<table>
<thead>
<tr>
<th>Hair Color</th>
<th>Age</th>
<th>4 wk</th>
<th>6 wk</th>
<th>10 wk</th>
<th>12 wk</th>
<th>14 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>Inorganic Se</td>
<td>.73</td>
<td>.92</td>
<td>.78</td>
<td>.78</td>
<td>.80</td>
</tr>
<tr>
<td>Red</td>
<td></td>
<td>.92</td>
<td>.92</td>
<td>.94</td>
<td>.94</td>
<td>.90</td>
</tr>
<tr>
<td>White</td>
<td>Organic Se</td>
<td>.80</td>
<td>.93</td>
<td>.94</td>
<td>.98</td>
<td>.95</td>
</tr>
<tr>
<td>Red</td>
<td></td>
<td>.89</td>
<td>.84</td>
<td>.97</td>
<td>.95</td>
<td>.97</td>
</tr>
</tbody>
</table>

Table 20. Coefficient of correlation between dietary selenium level and hair selenium concentration of grower-finisher pigs
<table>
<thead>
<tr>
<th>Item</th>
<th>Se Source</th>
<th>Se Level, ppm</th>
<th>Hair Color</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inorganic</td>
<td>Organic</td>
<td>0.3</td>
</tr>
<tr>
<td>No. of pigs</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Se, ppm</td>
<td>4.32</td>
<td>3.41</td>
<td>.89</td>
</tr>
<tr>
<td>Essential amino acid, % (DM basis)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>10.45</td>
<td>10.38</td>
<td>.12</td>
</tr>
<tr>
<td>His</td>
<td>1.14</td>
<td>1.19</td>
<td>.06</td>
</tr>
<tr>
<td>Ile</td>
<td>3.41</td>
<td>3.60</td>
<td>.19</td>
</tr>
<tr>
<td>Leu</td>
<td>6.26</td>
<td>6.29</td>
<td>.19</td>
</tr>
<tr>
<td>Lys</td>
<td>3.48</td>
<td>3.39</td>
<td>.36</td>
</tr>
<tr>
<td>Met</td>
<td>.52</td>
<td>.56</td>
<td>.04</td>
</tr>
<tr>
<td>Phe</td>
<td>2.49</td>
<td>2.53</td>
<td>.19</td>
</tr>
<tr>
<td>Thr</td>
<td>5.86</td>
<td>6.16</td>
<td>.17</td>
</tr>
<tr>
<td>Val</td>
<td>5.35</td>
<td>5.64</td>
<td>.17</td>
</tr>
<tr>
<td>Nonessential amino acids, % (DM basis)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>3.98</td>
<td>4.07</td>
<td>.06</td>
</tr>
<tr>
<td>Asp</td>
<td>4.41</td>
<td>6.09</td>
<td>.18</td>
</tr>
<tr>
<td>Cys</td>
<td>15.39</td>
<td>15.49</td>
<td>.82</td>
</tr>
<tr>
<td>Glu</td>
<td>10.94</td>
<td>8.85</td>
<td>1.35</td>
</tr>
<tr>
<td>Gly</td>
<td>4.06</td>
<td>4.25</td>
<td>.08</td>
</tr>
<tr>
<td>Hypro</td>
<td>.09</td>
<td>.09</td>
<td>.01</td>
</tr>
<tr>
<td>Pro</td>
<td>8.88</td>
<td>9.53</td>
<td>.29</td>
</tr>
<tr>
<td>Ser</td>
<td>8.06</td>
<td>8.54</td>
<td>.24</td>
</tr>
<tr>
<td>Tyr</td>
<td>3.20</td>
<td>3.33</td>
<td>.11</td>
</tr>
</tbody>
</table>

*Hair color response (P < .05).*

Table 21. Main effects of dietary selenium source, selenium level, and hair color on hair selenium and amino acid composition of hair.
<table>
<thead>
<tr>
<th>Se source :</th>
<th>Inorganic</th>
<th></th>
<th>Organic</th>
<th></th>
<th></th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se level :</td>
<td>3</td>
<td>7</td>
<td>3</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair color:</td>
<td>White</td>
<td>Red</td>
<td>White</td>
<td>Red</td>
<td>White</td>
<td>Red</td>
</tr>
<tr>
<td>Hair Se, ppm</td>
<td>.74</td>
<td>.76</td>
<td>5.13</td>
<td>10.65</td>
<td>.97</td>
<td>.73</td>
</tr>
<tr>
<td>Essential amino acid, % (DM basis)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>10.22</td>
<td>10.95</td>
<td>10.47</td>
<td>10.18</td>
<td>10.80</td>
<td>10.07</td>
</tr>
<tr>
<td>His</td>
<td>1.07</td>
<td>1.27</td>
<td>1.13</td>
<td>1.13</td>
<td>1.31</td>
<td>1.13</td>
</tr>
<tr>
<td>Ile</td>
<td>2.99</td>
<td>3.84</td>
<td>3.36</td>
<td>3.46</td>
<td>3.79</td>
<td>2.90</td>
</tr>
<tr>
<td>Lys</td>
<td>3.24</td>
<td>3.97</td>
<td>3.44</td>
<td>3.28</td>
<td>3.76</td>
<td>3.01</td>
</tr>
<tr>
<td>Met</td>
<td>48</td>
<td>56</td>
<td>46</td>
<td>.58</td>
<td>.55</td>
<td>.57</td>
</tr>
<tr>
<td>Phe</td>
<td>2.05</td>
<td>2.81</td>
<td>2.52</td>
<td>2.58</td>
<td>2.70</td>
<td>2.07</td>
</tr>
<tr>
<td>Thr</td>
<td>5.54</td>
<td>6.50</td>
<td>5.59</td>
<td>5.82</td>
<td>6.37</td>
<td>5.58</td>
</tr>
<tr>
<td>Val</td>
<td>5.09</td>
<td>5.72</td>
<td>5.21</td>
<td>5.40</td>
<td>5.64</td>
<td>5.00</td>
</tr>
<tr>
<td>Nonessential amino acid, % (DM basis)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>3.91</td>
<td>4.16</td>
<td>3.92</td>
<td>3.93</td>
<td>3.97</td>
<td>4.00</td>
</tr>
<tr>
<td>Asp</td>
<td>6.54</td>
<td>6.03</td>
<td>6.38</td>
<td>6.73</td>
<td>6.31</td>
<td>6.57</td>
</tr>
<tr>
<td>Cys</td>
<td>15.18</td>
<td>13.74</td>
<td>16.44</td>
<td>16.22</td>
<td>15.37</td>
<td>15.77</td>
</tr>
<tr>
<td>Gly</td>
<td>3.90</td>
<td>4.57</td>
<td>4.07</td>
<td>3.71</td>
<td>4.28</td>
<td>4.07</td>
</tr>
<tr>
<td>Hyp</td>
<td>.11</td>
<td>.09</td>
<td>.09</td>
<td>.10</td>
<td>.09</td>
<td>.11</td>
</tr>
<tr>
<td>Pro</td>
<td>7.97</td>
<td>10.72</td>
<td>8.93</td>
<td>7.92</td>
<td>9.85</td>
<td>8.28</td>
</tr>
<tr>
<td>Ser</td>
<td>7.94</td>
<td>8.86</td>
<td>7.89</td>
<td>7.55</td>
<td>8.93</td>
<td>8.03</td>
</tr>
<tr>
<td>Tyr</td>
<td>2.96</td>
<td>3.61</td>
<td>3.14</td>
<td>3.10</td>
<td>3.45</td>
<td>2.90</td>
</tr>
</tbody>
</table>

a Dietary Se source x hair color interaction response (P < .05).
b Dietary Se source x hair color interaction response (P < .01).
c Dietary Se source x Se level interaction response (P < .05).
d Dietary Se source x Se level interaction response (P < .01).
e Dietary Se source x Se level x hair color interaction response (P < .05).

Table 22. Treatment effects of dietary selenium source, selenium level and hair color on selenium concentration and amino acids composition of hair.
<table>
<thead>
<tr>
<th>Item</th>
<th>Hair Color</th>
<th>SEM</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White</td>
<td>Red</td>
<td>Black</td>
</tr>
<tr>
<td>No. of samples</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Se, ppm</td>
<td>.722&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.815&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.487&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diameter, mm</td>
<td>.18</td>
<td>.19</td>
<td>.18</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Means within a row with different superscripts differ (P < .05)

Table 23. Main effects of hair color and location on hair selenium concentration and hair diameter of gilts
Figure 10. The morphological differences in hair strands (top of hair) of white and black colored hair (A = white colored hair, B = black colored hair)
OVERALL CONCLUSIONS AND IMPLICATIONS

From several centuries ago, Se toxicity was recognized in various animal species and much of the current toxic Se levels has been established largely based upon the use of inorganic Se experiment.

The toxic level of inorganic Se when provided from sodium selenite was 5 to 10 ppm which was similar to previous results in grower-finisher pigs. However, pigs were less susceptible to organic Se compared with inorganic Se at the same levels. Consequently, toxic level of organic Se was approximately 15 ppm in grower-finisher pigs. Pigs were fed high levels of Se, also had a higher excretion of several essential micro-minerals which could cause a secondary mineral deficiency as well as Se toxicity.

Toxic symptoms of Se obtained in these studies (i.e. hair loss, hoof separation) were more severe in white haired pigs compared with red or black colored pigs. Selenium concentration was lower in the hair of white haired pigs than colored hair pigs.

When primiparous sows were fed 10 ppm organic Se for a long period, Se accumulated in the body which caused detrimental effects on reproductive performance even though 10 ppm organic Se was not toxic in grower-finisher pigs.

Tissue and serum Se concentrations of sows were higher when sows were fed organic Se from the beginning of the grower phase through lactation. High labile Se (in liver) increased Se transfer though the placenta and milk from dam to litter during gestation and lactation. This increased the Se status of progeny.

Hair Se concentration can be used as an indicator of Se status when pigs were fed high levels of Se. Colored hair contained more Se as dietary Se level increased. In addition, hair Se
concentrations were higher in the lower body areas than the upper body areas perhaps due to the hair being contaminated by urine or feces. Consequently, the hair sample should be collected from upper body areas (i.e. shoulder, back) rather than lower body areas.
APPENDIX

SELENIUM DETERMINATION PROCEDURE


1. Weigh samples and measure standard into 25 x 200 mm screw cap culture tubes.
   - Kidney – cut the edge of cortex.
   - Feces, grain or diet samples (high Fe) should be predigested in HNO₃ for 3 days.

2. Add boiling stones to culture tubes.

3. Add 10 mL HClO₄/HNO₃ mixed solution (4 HClO₄ : 1 HNO₃).

4. Heat tubes to 210°C in block digester. For approximately 4 hrs or white fumes are refluxing in the culture tube.

5. Remove tubes to wire rack to cool.

6. Once cool, add 1.0 mL HCl and swirl.

7. Heat tubes for 30 min at 150 °C to reduce Se (VI) ↔ Se (IV).

8. Remove tubes from heat, allow to cool.

9. Add 15 mL of .1 M EDTA, 5 mL cyclohexane and 2 mL of DAN solution.
   *Preparation the DAN solution: .1 g of 2,3-diaminonaphthalene, 99% (Sigma Co., St. Louis, Mo) with 100 mL of .1 M HCl.

10. Vortex each test tube for 5 second.
11. Incubate test tubes in a 60 °C for 45 min.

12. After incubation, vortex each test tube for 60 seconds.

13. Extract cyclohexane layer into fluorometer cuvettes and read on fluorometer.

   Use 369 nm excitation and 525 nm emission.
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