

THE EFFECTS OF INORGANIC AND ORGANIC SELENIUM SOURCES ON  
GROWTH PERFORMANCE, CARCASS TRAITS, TISSUE MINERAL  
CONCENTRATIONS, AND ENZYME ACTIVITY IN POULTRY

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## ABSTRACT

The purpose of this research was to compare dietary inorganic and organic Se sources in poultry. An experiment was conducted to assess daily egg production and Se deposition in eggs of hens fed diets supplemented with inorganic or organic Se. The results from this experiment indicated that hen production was not affected by Se source, and organic Se increased egg Se concentrations more than inorganic Se. Two experiments then were conducted with broilers to compare dietary organic and inorganic Se on growth performance, carcass traits, breast and plasma Se concentrations, and plasma glutathione peroxidase activity. The results from these experiments indicate that organic Se increases tissue Se concentration more than inorganic Se, but plasma glutathione peroxidase activity, growth performance, and carcass traits were not affected by source. Finally, three experiments were conducted to develop a Se-deficient diet, and then to compare plasma glutathione peroxidase and plasma and tissue Se concentrations in broilers fed a Se-deficient diet after they had been fed diets supplemented with either inorganic or organic Se. The results from these experiments indicate that organic Se increases tissue Se concentration compared with inorganic Se, but plasma glutathione peroxidase activity and growth performance were not affected by source. However, when broilers were placed on the Se deficient diet, organic Se maintained plasma glutathione peroxidase activity longer and at a higher level than inorganic Se. Overall, the results from these experiments indicate that dietary inorganic and organic Se result in similar growth performance and plasma glutathione peroxidase activity in broilers and layers, but organic Se increases tissue and egg Se concentrations more than inorganic Se.

## CHAPTER 1

### INTRODUCTION

Selenium (Se) is a dietary essential trace mineral for poultry (NRC, 1994). It was discovered in 1817 by Berzelius (Levander, 1986; Sunde, 1997), and for many years, Se was thought to be toxic to animals. However, in 1957, Se was reported to prevent liver necrosis in rats (Schwarz and Foltz, 1957), which established Se as a dietary essential nutrient. Since then, Se has been identified to be an integral part of over 30 distinct selenoproteins, including the enzyme, glutathione peroxidase (Sunde, 1997; Arthur, 2000).

The glutathione peroxidases are a group of antioxidant enzymes that are essential for protection of the cells of the body from peroxidative and free-radical damage (Sunde, 1997; Arthur, 2000). These enzymes are unique because Se is required in the form of selenocysteine (Rotruck et al., 1973; Sunde, 1997; Arthur, 2000). Selenium also is necessary in the diets of poultry to protect them from exudative diathesis and pancreatic fibrosis, which are two common conditions in poultry caused by Se deficiency (Cantor, 1975a,b).

Despite the establishment of a dietary need for Se, it still is considered to be the most toxic dietary essential trace mineral. Therefore, the FDA regulates supplementation of Se into poultry diets (FDA, 2000; AAFCO, 2003). The dietary requirement of poultry for Se often can be met by natural feedstuffs in the diet, but these feedstuffs vary widely in Se concentration depending on the region that they are grown. Therefore, it is common practice in poultry production in the U.S. to supplement diets with Se. One of the most common supplements used is sodium

selenite (SS), an inorganic form of Se. However, in 2000, the FDA approved the use of an organic source of Se, Se-enriched yeast (SY), in poultry diets (FDA, 2000). Therefore, the objectives of this research were to compare inorganic and organic Se sources to assess their effects on growth performance, carcass traits, daily egg production, egg quality, Se uptake in various tissues and plasma, and plasma glutathione peroxidase activity in broilers and laying hens.

**CHAPTER 2**  
**REVIEW OF LITERATURE**  
**HISTORY OF SELENIUM**

The Swedish chemist, Jons Jakob Berzelius discovered Se in 1817 in the flue dust of iron pyrite burners (Levander, 1986; Sunde, 1997). Selenium was identified in association with tellurium, which was named after the Latin term, tellus, for earth; so Se was named Se after the Greek term, selene, for moon. Since its discovery, Se has had an interesting history. In the 1930's, several researchers identified Se toxicity to be a direct cause of alkali disease and blind staggers (Franke, 1934a,b; Franke and Potter, 1935; Moxon, 1937), and then Nelson et al. (1943) classified Se as a carcinogen. However, in 1957, Schwarz and Foltz identified Se to be one of three compounds that prevented liver necrosis (vitamin E and cystine were the others), thus establishing Se as a nutritionally essential trace mineral. Furthermore, Rotruck et al. (1973) indicated that Se was essential for the proper function of the glutathione peroxidase enzyme, further establishing Se as nutritionally essential. This discovery identified one of Se's most important biochemical roles, and this role in glutathione peroxidase will be covered more in-depth later in this review.

**CHEMISTRY OF SELENIUM**

Selenium, atomic number 34 on the periodic table of elements, is a member of Group VIA along with oxygen, sulfur, tellurium, and polonium (Sunde, 1997), and it is classified as a metalloid element, indicating that it has both metallic and nonmetallic properties. Its natural atomic weight is 78.96, and Se has four common oxidative states: selenide (-2), Se (0), selenite or selenious acid (+4), and selenate

or selenic acid (+6). Selenium has six naturally occurring stable isotopes with the  $^{78}\text{Se}$  and  $^{80}\text{Se}$  forms accounting for over 73% of the total isotopes.

Because of their positions on the periodic table of elements, Se and sulfur, atomic number 16, have similar chemical properties, such as similar ionic and covalent bond lengths, and they also have similar electronegativities. These similarities can make it very difficult to chemically distinguish Se from sulfur compounds. However, these two elements can be easily distinguished under physiological conditions because  $\text{H}_2\text{Se}$  is a much stronger acid than is  $\text{H}_2\text{S}$ , and at physiological pH, selenocysteine is predominately deprotonated while cysteine is mostly protonated. Furthermore, selenious and selenic acids have much higher reduction potentials than do the sulfurous and sulfuric acids. These differences have significant implications in the biochemistry of Se.

### **SELENIUM DISTRIBUTION IN TISSUES**

Selenium can be found in all cells and tissues of the body, but the concentration of Se will depend on the chemical form and amount of Se provided by the diet. According to Behne and Wolters (1983) and Behne and Hofer-Bosse (1984), using rats supplemented with 0.30 ppm Se, the highest concentration of Se is in the kidneys, followed in descending order, by the testes, liver, adrenals, erythrocytes, plasma, spleen, pancreas, lungs, heart, thymus, gastrointestinal tract, skeleton, brain, and muscle. Sunde (1997) calculated total amounts of Se based on the data of Behne and Wolters (1983) and Behne and Hofer-Bosse (1984) and reported that the largest total amount of Se was in muscle followed by the liver,

plasma, erythrocytes, and kidneys. Schroeder et al. (1970) reported a similar distribution in samples taken from autopsies of North Americans.

Scott and Thompson (1971) reported that Se levels of the blood, muscle, liver, kidneys, and skin increased linearly in chicks fed up to 0.30 ppm Se from an inorganic Se source. They also reported that increasing Se to 0.80 ppm only resulted in higher levels of Se in the liver and kidneys with no significant increase in blood or muscle Se concentration. In contrast, muscle and blood Se levels were increased more using an organic Se source up to 0.67 ppm than an inorganic source at the same dietary levels (Scott and Thompson, 1971). Similarly, Latshaw (1975) reported increased muscle, liver, and egg Se levels when laying hens were fed an organic versus inorganic Se. Arnold et al. (1973) reported that the Se concentration of chicken feathers also was increased as dietary levels of Se increased from 0.30 to 8 ppm.

### **SELENIUM METABOLISM**

The metabolism of Se is dependent on its chemical form and on the amount ingested. However, the location in the gastrointestinal tract where Se is absorbed, regardless of Se source, seems to be consistent. Wright and Bell (1966), using sheep and pigs, and Whanger et al. (1976), using rats, agree that the majority of dietary Se is absorbed in the duodenum. Whanger et al. (1976) also indicated that there was some absorption of Se in the jejunum and ileum, but practically none from the stomach. However, there does seem to be differences in type of absorption depending on source. Combs and Combs (1986) indicated that inorganic sources of Se, such as SS or selenates, are passively absorbed, while organic sources, such

as SY or selenomethionine (SM), are actively absorbed via amino acid transport mechanisms.

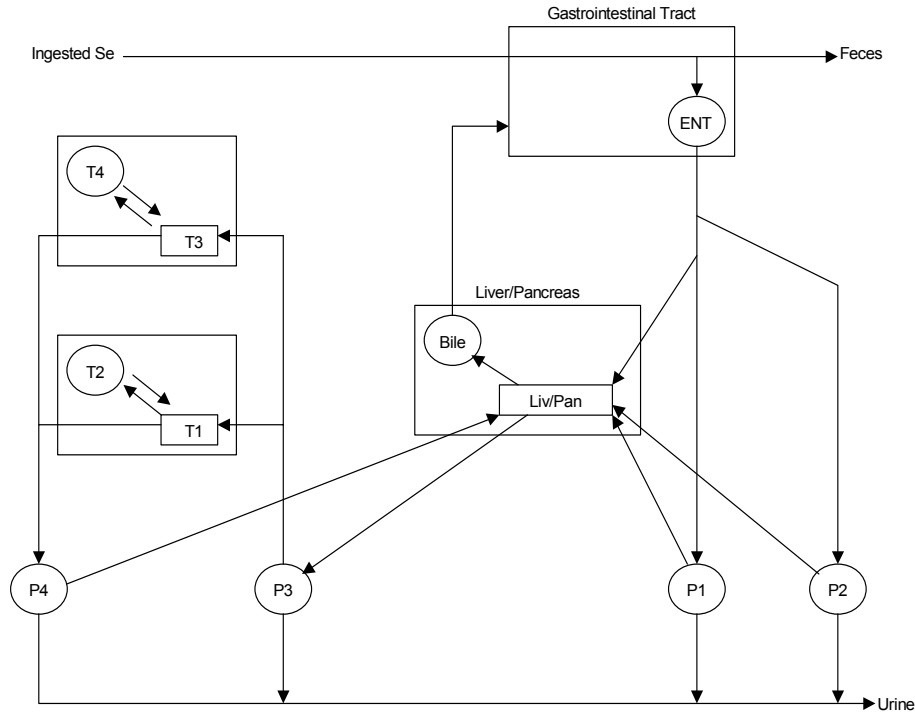


Figure 2.1. Kinetic model of selenium metabolism. Adapted from Sunde (1997) based on the model of Patterson et al. (1989) and Swanson et al. (1991).

A kinetic model of the flux of Se in human metabolism has been proposed by Patterson et al. (1989), Swanson et al. (1991), and Patterson and Zech (1992) and is shown above in Figure 2.1. In this model, there are several differences in the flux and metabolism of inorganic and organic Se. Inorganic forms of Se are absorbed from the gastrointestinal tract at a lower rate than organic forms, resulting in higher excretion of inorganic Se in the feces. After absorption, approximately 76% of the absorbed inorganic Se moves quickly (peak of 3 h) into a fast turnover ( $t_{1/2}$  of 20 min) plasma pool, while the remainder of the absorbed inorganic Se has a delayed

appearance (peak of 10 h) in a much slower turnover ( $t_{1/2}$  of 3 h) secondary plasma pool. Regardless of the plasma pool in which the inorganic Se is present, approximately 90% of the inorganic Se in each pool moves into the liver. After the liver, some of the inorganic Se returns to the gastrointestinal tract via the bile, while the rest moves into another slow turnover ( $t_{1/2}$  of 12 h) plasma pool. From this plasma pool, practically all of the inorganic Se moves into fast turnover tissues with over 70% of it being recycled in these tissues. The remaining inorganic Se in the tissues fluxes into a fourth plasma pool (peak of > 50 h and  $t_{1/2}$  of 6.6 d), where very little of it is returned to the liver and the rest is excreted via the urine.

There are several distinct differences in the flux of organic Se. First, over 95% of the organic Se is absorbed from the gastrointestinal tract. Once absorbed, the liver clears more than 50% of the organic Se immediately, which is similar to the fate of an amino acid. The remainder of the absorbed organic Se fluxes through two plasma pools before moving into the liver. Similar to inorganic Se, there is some recycling through the gastrointestinal tract via bile. After leaving the liver, organic Se enters a third plasma pool where one-half of the Se then will go to fast turnover tissues and the other half of the Se will go into slow turnover tissues, such as muscle. The organic Se from both tissue types will then enter the fourth plasma pool where almost all of it is recycled back to the liver, whereas almost all of the inorganic Se is excreted into the urine from the fourth plasma pool.

The currently proposed metabolic pathways for Se are shown below in Figure 2.2, and the pathways indicate that because of its reduction potentials, Se tends to be reduced when metabolized.

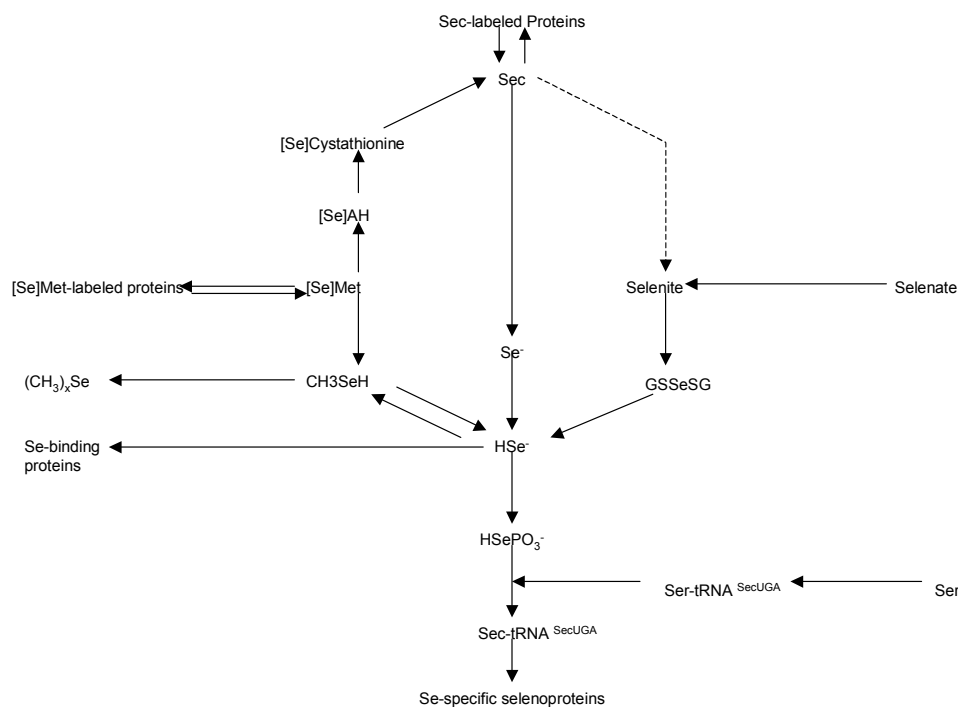


Figure 2.2. The proposed metabolic pathways of selenium. Adapted from Sunde (1997).

Inorganic Se sources are metabolized in the following manner as summarized by Sunde (1997). Axley and Stadtman (1989) reported that selenate first is converted to selenite. Then, selenite is nonenzymically reduced to elemental Se by glutathione forming seleno-diglutathione (GS-Se-SG; Ganther, 1966). In the absence of oxygen, seleno-diglutathione is further reduced to selenide ( $\text{HSe}^-$ ) by glutathione reductase (Hsieh and Ganther, 1975). At this point, selenide can have several different fates. It can be methylated to form methaneselenol ( $\text{CH}_3\text{SeH}$ ), which then can form dimethylselenide or trimethylselenonium ion ( $(\text{CH}_3)_x\text{SeH}$ ; Hsieh and Ganther, 1977). Selenide also can bind to the Se-binding proteins, or it can be a substrate for selenophosphate synthetase for the tRNA-mediated synthesis of

selenoproteins (Sunde, 1997). This last step converts inorganic Se into the organic forms of Se that are found in mammalian tissues.

Organic Se is metabolized differently than inorganic Se (Sunde, 1997). Dietary selenomethionine can be readily incorporated into protein ([Se]Met) as selenomethionine because it is esterified to methioninyl-tRNA only slightly less efficiently as Met (Hoffman et al., 1970; McConnell and Hoffman, 1972).

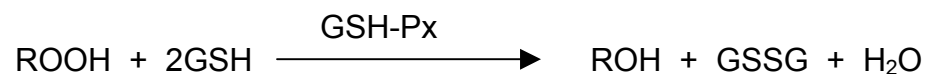
Selenomethionine can be metabolized to Se-adenosyl methionine (SeAM), and then to Se-adenosyl homocysteine (SeAH; Markham et al., 1980). The SeAH is readily converted to selenocysteine via cystathionine  $\beta$ -synthase and cystathionine  $\gamma$ -lyase. Selenocysteine then can be incorporated into proteins or degraded, releasing selenite, or it can be degraded by selenocysteine lyase, releasing elemental Se ( $\text{Se}^0$ ), which can be reduced to selenide (Esaki et al., 1982). Another potential fate for selenomethionine is to be transaminated to methaneselenol (Steele and Benevenga, 1979), and then methaneselenol can be transformed to selenide via S-methyltransferase (Sunde, 1997). At this point, selenide would be metabolized as discussed above.

### **GLUTATHIONE PEROXIDASES**

The processes of oxidation and reduction are necessary in the biochemistry of the body. This gain or removal of an electron keeps many of the life processes working. As respiration occurs in animals, which is defined as the process from which cells derive energy in the form of ATP from the reactions of hydrogen and oxygen, they often produce various peroxides. These peroxides, including hydrogen

peroxide, can be harmful to the body as they can lead to generation of free radicals, which can damage or destroy cells (Arthur, 2000).

The collective enzymes, known as the glutathione peroxidases (GSH-Px), are hydroperoxidases, and their function is to protect the body from these harmful peroxides (Arthur, 2000). The primary function of these enzymes is to catalyze a reaction that removes hydrogen peroxide from erythrocytes via reduced glutathione. The reduced glutathione is made via the enzyme, glutathione reductase, from oxidized glutathione. This reduction process also requires NADPH, which is provided by the pentose phosphate pathway. The general reaction for GSH-Px, as described by Rotruck et al. (1973), is shown below. In this schematic, ROOH is any hydrogen or lipid peroxide, GSH is glutathione in the reduced form, ROH is the reduced peroxide, GSSG is oxidized glutathione (Rotruck et al., 1973; Levander, 1986; Sunde, 1997).



Mills (1957) first described the activity of glutathione peroxidase, and it was hypothesized that its function was to protect red blood cells from oxidative hemolysis. In 1973, Rotruck et al. suggested that Se was an integral part of glutathione peroxidase, which Flohe et al. (1973) later demonstrated. Since that time, six different glutathione peroxidase enzymes have been elucidated. Four of these require selenocysteine at their active site for proper function, while the other two only require cysteine at their active site and thus, are not Se-dependent. The

following discussion will focus on the four Se-dependent glutathione peroxidase enzymes.

The GSH-Px that Mills described in 1957 is the classical cytosolic glutathione peroxidase (GPX-1) present in all cells of the body. It can metabolize hydrogen peroxide and several organic peroxides, such as cholesterol and long-chain fatty acid peroxides (Sunde, 1997). The GPX-1 enzyme is a tetrameric protein with four identical subunits, and each subunit contains one selenocysteine (Arthur, 2000). This enzyme is very specific for glutathione as a reducing substrate, and as such it often is discussed in relation with glutathione reductase activity (Sunde, 1997; Arthur, 2000).

The second GPX enzyme (GPX-2) also occurs in the cytosol, and it also has a tetrameric structure (Sunde, 1997; Arthur, 2000). However, this enzyme is unique because it mainly is found in the gastrointestinal tract. The GPX-2 enzyme is similar in amino acid identity (66%) and nucleotide sequence identity (61%) to GPX-1 (Sunde, 1997). It also is specific to glutathione as a substrate, and it reduces hydrogen peroxide and fatty acid peroxides (Arthur, 2000). Esworthy et al. (1998) has hypothesized that the specific function of GPX-2 is to metabolize ingested lipid hydroperoxides.

Shortly after the discovery of GPX-1, Chow and Tappel (1974) indicated that GPX activity in plasma responded quickly to Se deficiency and resupplementation. This plasma GPX activity was originally thought to be due to GPX-1 leaking from the liver or other organs, but it did not react with antibodies to GPX-1 purified from red blood cells (Takahashi and Cohen, 1986). This lack of reactivity indicated that this

GPX was a distinct enzyme, and ultimately it was determined to be a glycoprotein with distinct extracellular functions (Sunde, 1997; Arthur, 2000). Similar to GPX-1, it has a tetrameric protein structure, but it shares only 40 to 50% amino acid homology to GPX-1 (Arthur, 2000). Furthermore, GPX-3 can metabolize phospholipid hydroperoxides, whereas GPX-1 cannot. The major source for GPX-3 is the kidney, and the mRNA for GPX-3 is predominately found in the proximal tubules. Most of the circulating GPX-3 is found in plasma and extracellular fluids, and it often is glycosylated to improve stability (Arthur, 2000). Although its role is not completely clear, GPX-3 is thought to protect cell membranes extracellularly from localized peroxide-induced damage (Arthur, 2000).

The fourth Se-dependent GPX enzyme (GPX-4) is significantly different from the GPX-1 enzyme. The GPX-4 has a monomeric protein structure compared with the tetrameric structure of GPX-1, and it is not glutathione specific, such that it can use phospholipid hydroperoxides as substrates (Sunde, 1997; Arthur, 2000). Because of its unique structure, GPX-4 is believed to be able to bind to a wider range of substrates than the other GPX enzymes. Although its function has not been clearly defined, Godeas et al. (1994) indicated that GPX-4 may protect cellular membranes from hydroperoxides via the cytosol by rolling along membrane surfaces and mitochondrial intermembrane spaces.

### **OTHER SELENOPROTEINS**

Besides the glutathione peroxidase family of enzymes, there are several other proteins that also must have Se in the form of selenocysteine to function properly. These include three forms of the deiodinase enzyme necessary for the formation of

the thyroid hormone, thyroxine; the plasma selenoprotein P; and the muscle selenoprotein W. The thyroxine 5'-deiodinase-1, 2, and 3 (DI1, DI2, and DI3) enzymes are responsible for deiodinating thyroxine or reverse triiodothyronine to make triiodothyronine or diiodothyronine, respectively (Sunde, 1997). More than 90% of the circulating triiodothyronine in the plasma is produced by DI1, which was first identified as a selenoenzyme by Arthur et al. (1990) and Behne et al. (1990). The other deiodinase enzymes, DI2 and DI3, are most prevalent in the central nervous system and brown adipose tissue (Sunde, 1997).

Hill et al. (1991) first described the protein known as plasma selenoprotein P. The physiological role of this protein is currently unknown. Originally, Motsenbocker and Tappel (1982) thought that this protein might be a Se transport protein, but since then, it has been postulated to be an antioxidant defense protein (Burk et al., 1991). A third hypothesis offered by Sunde (1997) is that selenoprotein P has a role in the disulfide exchange of cysteine-plus-selenocysteine. Pedersen et al. (1972) first reported the existence of a selenoprotein that was missing in lambs suffering from white muscle disease. This selenoprotein since has been identified as muscle selenoprotein W by Vendeland et al. (1995), but its direct role in preventing white muscle disease is not clearly defined.

### **SELENIUM DEFICIENCY IN POULTRY**

Although the requirement for Se often is met by the natural feedstuffs in poultry diets, there are several detrimental conditions that can result in poultry when dietary Se is deficient. Exudative diathesis, pancreatic fibrosis, and impaired reproduction are observed if the Se level in the diet is deficient. Exudative diathesis

and pancreatic fibrosis, which are discussed in detail below, have a major difference in the form of Se needed to alleviate their deficiency signs. Reproductive impairment, on the other hand, does not seem to be specific in the form of Se needed to alleviate its deficiency signs (Underwood and Suttle, 1999).

Exudative diathesis is characterized by a general edema due to atypical permeability of the capillary walls (Underwood and Suttle, 1999). It first appears on the breast, wing, and neck as greenish-blue discoloration due to fluid accumulation under the ventral skin. Abnormal growth rate and high mortality are common in flocks with exudative diathesis, and Hartley and Grant (1961) indicated that this condition usually occurs between 3 and 6 weeks of age. Noguchi et al. (1973a) reported that either Se or vitamin E could prevent exudative diathesis. In a subsequent study, Noguchi et al. (1973b) reported that dietary Se is directly related to GPX-3 activity and the prevention of exudative diathesis. Selenium in the form of SS or selenocysteine provides the most protection from exudative diathesis (Cantor et al. 1975a,b).

Pancreatic fibrosis results from a severe Se deficiency in poultry, and it causes atrophy of the pancreas, as well as poor growth and feathering (Thompson and Scott, 1969). Bunk and Combs (1980) reported that appetite depression associated with this condition is negated within hours of Se supplementation. Furthermore, Noguchi et al. (1973a) indicated that the pancreatic lesions, which become apparent by 6 days of age, return to normal within 2 weeks after the onset of Se supplementation. High dietary vitamin E cannot alleviate this condition as pancreatic fibrosis results in a secondary vitamin E deficiency due to impaired

formation of lipid bile micelles, which are necessary for the absorption of vitamin E (Thompson and Scott, 1969). Selenium in the form of SM protects poultry from pancreatic fibrosis more efficiently than SS or selenocysteine (Cantor et al. 1975a,b).

Impaired reproduction in females also can result from Se deficiency. Cantor and Scott (1974) reported that egg production and hatchability were reduced in laying hens fed diets with reduced levels of Se, and Latshaw et al. (1977) indicated that hatchability was the most sensitive criteria of Se deficiency in hens. Furthermore, Jensen (1968) reported that low dietary Se impaired fertile egg hatchability and chick viability in Japanese quail.

### **DIETARY SOURCES OF SELENIUM**

The dietary sources of Se for poultry can be divided into two groups: natural feedstuffs, such as corn or soybean meal, and supplemental sources, such as sodium selenite. The Se levels in plant-based feedstuffs vary greatly depending on plant species and soil status (Levander, 1986). Plants then can be divided into two groups based on their ability to accumulate Se. Examples of Se-accumulator plants are species of *Astragalus* and *Neptunia*, which can have Se levels well over 1,000 ppm (Levander, 1986). However, these Se-accumulator plants are not commonly used in animal diets. Selenium non-accumulator plants, such as corn, wheat, or oats, are more typical of the feedstuffs used for animal diets. Pastures and forages grown in areas where animals do not seem to have any Se-deficiency-related conditions usually have levels of 0.10 ppm Se or greater (Levander, 1986). However, areas where animals do express signs of these conditions often have Se

levels of 0.05 ppm or lower (Levander, 1986). There also is considerable difference in the Se level of grains, with the extremes being wheat containing 0.32 ppm of Se or oats containing 0.20 ppm of Se (Levander, 1986). Beilstein and Whanger (1986) indicated that the major form of Se in non-accumulator plants was SM. Peterson and Butler (1962) and Olson et al. (1970) indicated that Se existed in several forms, including SM, selenocysteine, or Se-methylselenomethionine, in typical forages and seeds consumed by animals. Natural feedstuffs made from animal products also contain Se, and the Se found in these sources is predominantly selenocysteine (Hawkes et al., 1985). Both Scott and Thompson (1971) and Gabrielsen and Opstvedt (1980b) reported that various fishmeals contained relatively high levels of Se, ranging from 1.3 to 6.2 ppm.

The supplemental sources of Se vary in their ability to meet the requirement of the animal due to chemical form, and how that form is metabolized once it is absorbed. Schwarz and Foltz (1957) divided these sources into three categories according to their potency against liver necrosis in rats. The first category contains elemental Se, which is poorly absorbed by the animal. The inorganic salts, such as selenites, selenates, and the Se analogs of methionine and cysteine, make up the second category. Originally, Schwarz and Foltz (1957) reported that there were no differences in the ability of these sources to protect against liver necrosis. However, as indicated above, Noguchi et al. (1973a,b) and Cantor et al. (1975a,b) indicated differences in their ability to alleviate Se-deficiency conditions within this group of compounds. The third category contains organic Se compounds that are more

active than those in the second group. These sources have not been well researched because of their chemical instability and low yields.

### **USE OF SELENIUM FOR POULTRY**

Selenium is a dietary essential nutrient for poultry (NRC, 1994). The Se requirement for the laying hen ranges from 0.05 to 0.08 ppm depending on daily feed intake while the broiler's requirement is 0.15 ppm (NRC, 1994). Natural feedstuffs often will meet these requirements, but as mentioned before, there is considerable variation in Se content of natural feedstuffs. Therefore, it is common practice in the poultry industry in the U.S. to supplement the diet with some form of Se. The maximum level of Se supplementation allowed in poultry diets is 0.30 ppm (NRC, 1994; AAFCO, 2003). This supplementation has historically come from inorganic sources of Se, primarily SS, but in 2000, the FDA approved the use of SY. There have been several reports comparing the use of organic Se with inorganic Se in broilers and laying hens, which will be discussed below.

### **SELENIUM IN THE DIETS OF BROILERS**

The response to dietary Se supplementation has been somewhat variable. Several researchers reported that Se supplementation increased growth performance (Thompson and Scott, 1969; Bunks and Combs, 1980; Cantor et al., 1982; Echevarria et al., 1988b) while several others have reported no effect (Miller et al., 1972; Shan and Davis, 1994; Edens et al., 2001; Spears et al., 2003). Only Echevarria et al. (1988a) reported a negative effect of Se on growth performance, and they were feeding very high levels of SS (3, 6, or 9 ppm), which could be toxic to

broilers. None of the research has reported a difference in growth performance due to source (organic versus inorganic).

The results of Se supplementation on tissue Se concentrations are fairly consistent when diets are supplemented with Se. There are several reports of Se supplementation increasing breast, liver, or plasma Se levels (Scott and Thompson, 1971; Cantor et al., 1982; Echevarria et al., 1988a,b; and Spears et al., 2003). Furthermore, Cantor et al. (1982) and Spears et al. (2003) both indicated that organic Se increased tissue Se levels more than inorganic Se or a diet with no supplemental Se.

The published results on plasma GPX-3 activity are variable. Cantor et al. (1982) and Spears et al. (2003) both reported that plasma GPX-3 activity was increased when diets were supplemented with Se, regardless of source. However, in a second trial, Spears et al. (2003) indicated that plasma GPX-3 was increased more by SS supplementation than by SM. Only Cantor et al. (1975) indicated no differences in plasma GPX-3 when broilers were fed SS, SM, or no supplemental Se.

### **SELENIUM IN THE DIETS OF LAYERS**

The effects on supplemental Se in diets for laying hens are relatively consistent. Several researchers have indicated no difference in daily egg production due to Se supplementation or source (Cantor and Scott, 1974; Latshaw and Osman, 1975; Ort and Latshaw, 1978; Cantor et al., 2000; and Patton, 2000).

The research on whole egg Se concentration when diets are supplemented with Se is vast and relatively consistent. The increase in whole egg Se when diets

are supplemented with Se has been reported by several authors and is very consistent (Cantor and Scott, 1974; Latshaw and Osman, 1975; Ort and Latshaw, 1978; Latshaw and Biggert, 1981; Martello and Latshaw, 1982; and Swanson, 1987; Davis et al., 1996; and Cantor et al., 2000). Several reports also have indicated that yolk or white Se concentrations are increased depending on Se source, but these reports are slightly variable. Latshaw and Osman (1975), Martello and Latshaw (1982), Swanson (1987), and Davis et al. (1996) reported that eggs from hens fed diets supplemented with SM had higher Se in the white than those fed SS or selenocysteine. Ort and Latshaw (1978) indicated that the Se level of yolks was greater when hens were fed SS, but Swanson (1987) and Davis et al. (1996) indicated that SM increased yolk Se more than SS. Latshaw and Biggert (1981) and Cantor et al. (2000) reported that whole egg, egg white, and egg yolk Se levels were greater in hens fed SM compared with those fed SS.

## **CONCLUSIONS**

Selenium is a dietary essential nutrient for poultry. It prevents exudative diathesis and pancreatic fibrosis, and it is necessary to maintain proper function of glutathione peroxidase. Selenium supplementation has been shown to increase tissue Se levels as well as glutathione peroxidase activity in poultry. It is the intent of the research contained herein to compare the effects of inorganic Se with organic Se sources in broilers and laying hens, and then to determine the fates of Se once it has been absorbed into various tissues.

## CHAPTER 3

### EFFECT OF INORGANIC VERSUS ORGANIC SELENIUM ON HEN PRODUCTION AND EGG SELENIUM CONCENTRATION

#### INTRODUCTION

Selenium is a dietary essential nutrient for laying hens (NRC, 1994). It is essential for proper function of the antioxidant enzyme, glutathione peroxidase, which protects the cell by destroying free radicals in the cell (Rotruck et al., 1973). The laying hen's requirement for Se ranges from 0.05 to 0.08 ppm depending on daily feed intake (NRC, 1994). This Se requirement can be met by a typical corn-soybean meal diet without additional supplementation. However, Se content of feed grains widely varies from region to region (NRC, 1994), and thus it is a common practice in the poultry industry to supplement laying hen diets. The maximum allowed Se inclusion level in the U.S. is 0.30 ppm. Historically, the Se source that has been used is the inorganic sodium selenite ( $\text{Na}_2\text{SeO}_3$ ; SS). However, in 2000, an organic source of Se was approved for use as a feed supplement in poultry diets (FDA, 2000). This organic source of Se is a Se-enriched yeast (SY) that is produced by growing the yeast-strain, *Saccharomyces cerevisiae*, in a high-Se medium (AAFCO, 2003). Beilstein and Whanger (1986) and Kelly and Power (1995) indicated that the majority of the Se in SY is selenomethionine (SM), a Se analog of methionine.

Research comparing SS with SM or SY in laying hens has been published, and the results from these experiments are consistent. Whole-egg Se concentration is increased by SS, SM, or SY supplementation as dietary level increases, but the

SM and SY have been reported to increase egg Se more than SS (Swanson, 1987; Davis et al., 1996; and Cantor et al., 2000). Arnold et al. (1973) reported an increase in whole-egg Se content when hens were fed 8 ppm of SS compared with hens fed diets containing none or 2 ppm of SS. Davis et al. (1996) indicated that both SS and SM fed at 2 ppm increased yolk Se concentrations compared to a diet not supplemented with Se; however, Arnold et al. (1973) did not report any difference in whole-egg Se content when hens were fed 2 ppm of SS compared to those fed a diet not supplemented with Se.

Despite the extensive research that has been conducted comparing SS with SM or SY, there has not been sufficient research conducted to directly compare titrated levels of SS with SY, which are two of the commercially- available Se supplements that are approved for use in poultry feeds. Furthermore, in the research cited above, the authors collected and analyzed eggs in either weekly or several week intervals over time. Therefore, the primary objective of our trial was to compare Se deposition in the whole egg when hens were fed diets supplemented with various levels of inorganic and organic sources of Se, specifically SS and SY, over a 28-d period. Our secondary objective was to determine if elevated levels of supplemental Se affected the performance of the laying hen.

## **MATERIALS AND METHODS**

### **General**

An experiment was conducted to compare the effects of an inorganic Se source with an organic Se source on daily egg production and whole-egg Se

concentrations in laying hens. The methods used were approved by the Louisiana State University Agricultural Center Animal Care and Use Committee.

Two hundred eighty-eight Hy-Line W-36 laying hens<sup>1</sup>, approximately 70 wk of age, were used for this 28-d experiment. A corn-soybean meal basal diet was formulated to provide 0.82% total lysine and 2,950 kcal/kg of ME to the laying hens (Table 3.1). The basal diet met or exceeded the other nutrient requirements suggested in the Hy-Line W-36 Commercial Management Guide (2003). The basal diet contained a trace mineral premix that provided no supplemental Se. The SS and SY<sup>2</sup> were supplemented into the basal diet at 0.15, 0.30, 0.60, or 3.00 ppm in a 2 x 4 factorial treatment arrangement, and the basal diet served as the 0 ppm level of supplemented Se. Each treatment was replicated four times. Each replicate consisted of two adjoining pens with four hens per individual pen for a total of eight hens per replicate.

The laying hens were housed in a commercial-type tunnel-ventilated house at the Louisiana State University Agricultural Center Poultry Farm for the duration of this experiment. The pens were galvanized-metal wire (approximately 52 cm x 34 cm x 30 cm) in double-decker rows. For this experiment, one row of high cages and one row of low cages were used. Each pen had one nipple-waterer. A continuous, galvanized-metal feed trough was divided by replicate to eliminate the possibility of cross-contamination of feed and to insure that the hens were not able to consume the adjoining replicate's feed. A wire divider also was inserted in the egg-collection area to prevent eggs from being mixed with another replicate's eggs. Water and

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<sup>1</sup> Hy-Line International, West Des Moines, IA

<sup>2</sup> Sel-Plex, Alltech, Inc. Nicholasville, KY

Table 3.1. Composition of basal diet, as-fed basis<sup>1</sup>

Ingredient	%
Corn	62.01
Soybean meal (47.5% CP)	22.25
Limestone	9.90
Tallow	3.84
Monocalcium phosphate	1.40
Salt	0.35
Vitamins <sup>2</sup>	0.05
DL-Methionine	0.09
Trace minerals <sup>3</sup>	0.10
Calculated composition	
ME, kcal/kg	2,950
Crude fat, %	3.09
Crude protein, %	15.77
Lysine, %	0.82
TSAA, %	0.62
Calcium, %	4.10
Phosphorous, %	0.62
Non-phytate phosphorous, %	0.40
Selenium, mg/kg	0.04

<sup>1</sup>A 1% basal premix was made with the selenium products for mixing of dietary treatments.

<sup>2</sup>Provides per kilogram of diet: vitamin A, 8,000 IU; vitamin D<sub>3</sub>, 3,000 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B<sub>12</sub>, 0.02 mg; biotin, 0.1 mg; folacin, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; and thiamin, 3 mg.

<sup>3</sup>Provides per kilogram of diet: copper (cupric sulfate pentahydrate), 10.00 mg; iodine (ethylenediamine dihydriodide), 1.00 mg; iron (ferrous sulfate monohydrate), 50.00 mg; manganese (manganese sulfate monohydrate), 60.00 mg; and zinc (zinc sulfate monohydrate), 60 mg.

feed in mash form were provided *ad libitum* throughout the experiment. The experiment was conducted in the fall season and the temperature and lighting schedules were similar to guidelines set in the Hy-Line W-36 Commercial Management Guide (2003).

Daily egg production was evaluated during the 28-d experiment. Each day at approximately 1200 h, the total eggs produced and the number of dirty, cracked, or shell-less eggs were recorded for each replicate. Total feed intake was measured from d 0 to 28 of the experiment. Feed efficiency for each hen was calculated as eggs produced per kilogram of feed consumed.

On d 0, 4, 8, 12, 16, 20, 24, and 28 of the experiment, two eggs per replicate were randomly selected for whole-egg Se analysis. These eggs were marked with their treatment and replicate pen number and then stored in a cooler at 7.2°C until they were homogenized. The eggs were cracked and the shells were discarded. The fluid eggs then were homogenized together with a malt blender and stored frozen in 12 mL plastic cups until Se analysis could be conducted. The eggs collected on d 24 and 28 also were used for determination of albumin quality before homogenization. Albumin quality was determined by measuring the Haugh units of each egg using the methods of Brant et al. (1951). To examine the temperature effects on albumin quality, the d-24 collection was stored in a cooler at 7.2°C and the d-28 collection was stored at 22.2°C for 3 d before Haugh unit measurement.

### **Diet and Egg Selenium Analysis**

The homogenized eggs, diets, and SY and SS Se sources were analyzed for Se by the methods of Brown and Watkinson (1977) using a semi-automated

fluorimeter<sup>3</sup> at the Oregon State University Forage Analytical Service (Tables 3.2 and 3.3). All samples were wet digested in nitric and perchloric acids.

Table 3.2. Comparison of anticipated with actual analyzed selenium levels in diets

Level, mg/kg	0	0.15	0.30	0.60	3.00
Treatments:					
1. Basal	0.10	--	--	--	--
2. Sodium selenite	--	0.24	0.39	0.57	2.60
3. Selenium-enriched yeast	--	0.29	0.46	0.79	2.94

### Statistical Analysis

The daily egg production, albumin quality, and feed intake data were analyzed by analysis of variance procedures (Steel and Torrie, 1980) appropriate for a completely randomized design by the GLM procedure of SAS<sup>®</sup> (1995). The whole-egg Se concentration data were analyzed as repeated measures using the MIXED procedure of SAS<sup>®</sup> (1995). For this analysis, the unstructured covariance structure and Kenward-Roger degrees of freedom method were used. The replicate of layers served as the experimental unit for all analyses. Linear and quadratic orthogonal polynomial contrasts appropriate for unequally spaced treatments were determined using the IML procedure of SAS<sup>®</sup> (1995) to evaluate the effects of SY and SS. Contrast statements for source, level, and source by level linear and quadratic were conducted.

<sup>3</sup> Model 300 with 321 fluorimeter and 303A selenium cartridge, Astoria-Pacific International, Clackamas, OR

## RESULTS AND DISCUSSION

Egg percentage and percentage of shell-less eggs were not affected by source or level of Se ( $P > 0.05$ ; Table 3.4). However, the percentages of dirty and cracked eggs were higher from hens fed SY compared with those fed SS ( $P < 0.04$ ). Feed intake was not affected by source of Se ( $P > 0.05$ ), but it was increased in hens fed the basal diet compared to those supplemented with Se ( $P < 0.01$ ). Feed efficiency (eggs per kilogram of feed) was not affected by Se source ( $P > 0.05$ ), but there was a tendency (linear,  $P = 0.09$ ) for SS to increase efficiency. Albumin quality of eggs stored at 22.2°C was improved in hens fed SS compared with those fed SY ( $P < 0.04$ ), but albumin quality was not affected by diet in eggs stored at 7.2°C ( $P > 0.05$ ). The eggs from hens fed diets supplemented with Se, regardless of level or source, tended to have better albumin quality ( $P = 0.10$ ) at both temperatures compared with eggs from hens fed the basal diet.

Our results for egg production agree with those of Cantor et al. (2000) and Patton (2000) who reported no difference in egg production when hens were fed a basal diet supplemented with 0 or 0.30 ppm of SS or SY. However, our results disagree with those of Cantor and Scott (1974) who reported an increase in egg production for hens fed 0.10 ppm of SM relative to the 0 level of supplementation with egg production of hens fed SS at 0.10 ppm intermediate between the two.

Reduced egg production and feed intake are two signs of Se toxicity in laying hens. Arnold et al. (1973) reported a decrease in egg production when hens were supplemented with 8 ppm of SS but not when supplemented at 2 ppm of SS. Ort and Latshaw (1978) reported no adverse effects on egg production in hens

Table 3.3. Effect of selenium source on egg selenium concentration-overall<sup>1</sup>

Treatment	Se, mg/kg <sup>2a,b,c</sup>
1) Basal	0.249
2) 0.15 ppm SS	0.284
3) 0.30 ppm SS	0.299
4) 0.60 ppm SS	0.327
5) 3.00 ppm SS	0.641
6) 0.15 ppm SY	0.366
7) 0.30 ppm SY	0.495
8) 0.60 ppm SY	0.670
9) 3.00 ppm SY	2.207
PSEM	0.067

<sup>1</sup>Egg selenium concentration data are means of four (eight hens per replicate) replicates. Two eggs per replicate were collected every 4 d for the duration of the experiment. The experiment lasted 28 d. SS = sodium selenite; SY = selenium-enriched yeast.

<sup>2</sup>Selenium values reported in this column are the overall selenium concentrations for each treatment for the 28-d experiment.

<sup>a</sup>Treatment effect,  $P < 0.01$ .

<sup>b</sup>Time effect,  $P < 0.01$ .

<sup>c</sup>Treatment x time effect,  $P < 0.01$ .

Table 3.4. Effect of selenium source on daily egg production<sup>1</sup>

Treatment	Eggs, %	Dirty, % <sup>a</sup>	Cracked, % <sup>a</sup>	Shell- less, %	Eggs per kg Feed <sup>b</sup>	ADFI, g <sup>c</sup>	7.2°C Haugh <sup>2,d</sup>	22.2°C Haugh <sup>2,a,d</sup>
1) Basal	82.25	5.00	0	0	7.77	106.05	52.52	59.43
2) 0.15 ppm SS	77.50	3.50	0.50	0	7.92	97.57	60.38	64.44
3) 0.30 ppm SS	79.00	4.00	0.25	0	8.21	96.25	59.22	63.87
4) 0.60 ppm SS	80.75	3.25	0.25	0	7.87	102.85	58.29	58.92
5) 3.00 ppm SS	81.50	5.50	0.75	0.25	8.50	96.45	56.50	56.76
6) 0.15 ppm SY	77.00	8.50	1.50	0	7.88	98.00	57.18	55.77
7) 0.30 ppm SY	78.50	6.75	1.75	0	8.04	97.70	61.93	51.21
8) 0.60 ppm SY	81.00	5.25	1.25	0.25	8.08	100.23	56.64	60.76
9) 3.00 ppm SY	80.50	5.25	0.50	0	8.07	99.93	54.40	56.97
PSEM	2.32	1.04	0.50	0.12	0.28	2.45	3.04	3.04

<sup>1</sup>Egg performance data are means of four (eight hens per replicate) replicates. The experiment lasted 28 d. ADFI = average daily feed intake; SS = sodium selenite; SY = selenium-enriched yeast.

<sup>2</sup>Haugh units were determined using the method described by Brant et al. (1951). The 7.2°C Haugh units were measured on eggs collected on d-24, and the 22.2°C Haugh units were measured on eggs collected on d-28.

<sup>a</sup>Source,  $P < 0.04$ .

<sup>b</sup>SS linear,  $P = 0.09$ .

<sup>c</sup>Basal vs. selenium supplementation,  $P < 0.01$ .

<sup>d</sup>Basal vs. selenium supplementation,  $P = 0.10$ .

supplemented with 0, 0.1, 1, 3, 5 or 7 ppm of SS, but egg production was decreased by 9 ppm of SS. Cantor et al. (1984) indicated that the upper limit before feed intake decreases is 4 mg of SS per liter of drinking water, which is equivalent to 7 ppm of SS in the diet. Our feed intake and egg production results are in agreement with Cantor and Scott (1974), Cantor et al. (2000), and Patton (2000), and indicate that 3 ppm of SS or SM is not toxic to laying hens.

Roland (1988) reported that there is an average loss of 1.6% in revenue due to cracked or shell-less eggs in the table-egg industry, and he estimated this loss to cost the industry 55.5 million dollars per year. Furthermore, Roland (1988) attributed an additional 6.1% loss to uncollectable eggs, such as those that fall through the cages into the manure, which is estimated to cost 211.6 million dollars per year. In our experiment, the occurrence of cracked eggs was over 250% greater from hens supplemented with SY compared with those supplemented with SS. Our results, however, are not consistent with the published data. Siske et al. (2000) reported increased egg-shell strength when organic forms of Mn, Se, and Zn were substituted into the diet for one-half of the inorganic forms of these minerals. Patton (2000) reported no difference in egg breaking strength from hens fed supplemental SS or SY in two experiments, but an increase in egg breaking strength from hens supplemented with SY relative to SS in a third experiment.

Patton (2000) reported that SS or SY supplementation of 0.30 ppm had no effect on Haugh unit values in eggs on d 0, 21, or 42 compared with eggs from hens fed the basal diet. However, in a second experiment, SS supplementation at 0.10, 0.20, or 0.30 ppm improved Haugh units compared with eggs from hens fed SY at

the same dietary levels (Patton, 2000). The results of Patton (2000) and our experiment contradict those of Wakebe (1988) who reported that SY reduced the deterioration of the albumin after the egg is laid, which would slow carbon dioxide loss and maintain albumin quality. Our results also disagree with Arnold et al. (1973) who reported that Haugh units were not improved by SS supplementation.

We cannot fully explain why our results for cracked egg percentage and albumin quality differ from the published results, but we do have a possible explanation. Beilstein and Whanger (1986) and Kelly and Power (1995) indicated that the majority of the Se in SY is in the form of selenomethionine. Before these reports, Combs and Combs (1985) suggested that organic Se sources, such as SM or SY, were actively absorbed and then directly incorporated into protein. Our results, along with several others (Cantor and Scott, 1974; Swanson, 1987; and Cantor et al., 2000) indicate that SM is deposited to a greater extent in the egg than SS. If SM is deposited into the egg directly as SM, then it is possible that the Se in SM would not be available, or at least not immediately available, for incorporation into glutathione peroxidase. Selenocysteine is the form of Se that is incorporated into glutathione peroxidase (Forstrom et al., 1978). Sunde and Hoekstra (1980) reported that inorganic SS was efficiently metabolized into selenocysteine; however, Henry and Ammerman (1995) indicated that SM is converted into selenocysteine at a slower rate. The conversion of SS to selenocysteine also may occur before deposition in the egg as SS is metabolized in the liver, which is where the yolk proteins are deposited into the egg. Coincidentally, the yolk proteins are higher in SS than are the white proteins (Ort and Latshaw, 1978). Therefore, if SS is

converted to selenocysteine before deposition into the egg, then the selenocysteine in the egg may be more readily available for incorporation into glutathione peroxidase. This increase in glutathione peroxidase would act to protect the egg from damage by free-radicals, thus potentially creating a stronger and healthier egg. Selenium concentrations in the egg was increased linearly as SS or SY supplementation increased ( $P < 0.01$ ; Table 3.3). Eggs from hens fed the Se sources were higher in Se content than those from hens fed the basal diet ( $P < 0.01$ ), and eggs from hens fed SY were higher in Se than those from hens fed SS ( $P < 0.01$ ; Figure 3.1), which resulted in a source by level linear interaction. Whole-egg Se concentrations increased at each collection period with eggs from hens fed SY having higher Se concentrations than those from hens fed SS ( $P < 0.01$ ). The whole-egg selenium analysis results in our trial are in agreement with several other researchers. Cantor and Scott (1974), Ort and Latshaw (1978), Swanson (1987), Davis et al. (1996), Cantor et al. (2000), and Patton (2000) reported that dietary selenium supplementation increased egg selenium concentration, and the increase was generally proportional to the level of dietary supplementation. Also, similar to our results, these same researchers reported that organic selenium, whether from SM or SY resulted in a greater increase in egg selenium content than SS.

A reason for the increased Se deposition in the egg by SY is that SY is predominantly selenomethionine (Beilstein and Whanger, 1986; Kelly and Power, 1995). Ochoa-Solano and Gitler (1968) and Latshaw and Biggert (1981) indicated that selenomethionine could be incorporated into egg as effectively as methionine. Combs and Combs (1986) reported that organic Se sources, such as SM or SY, are

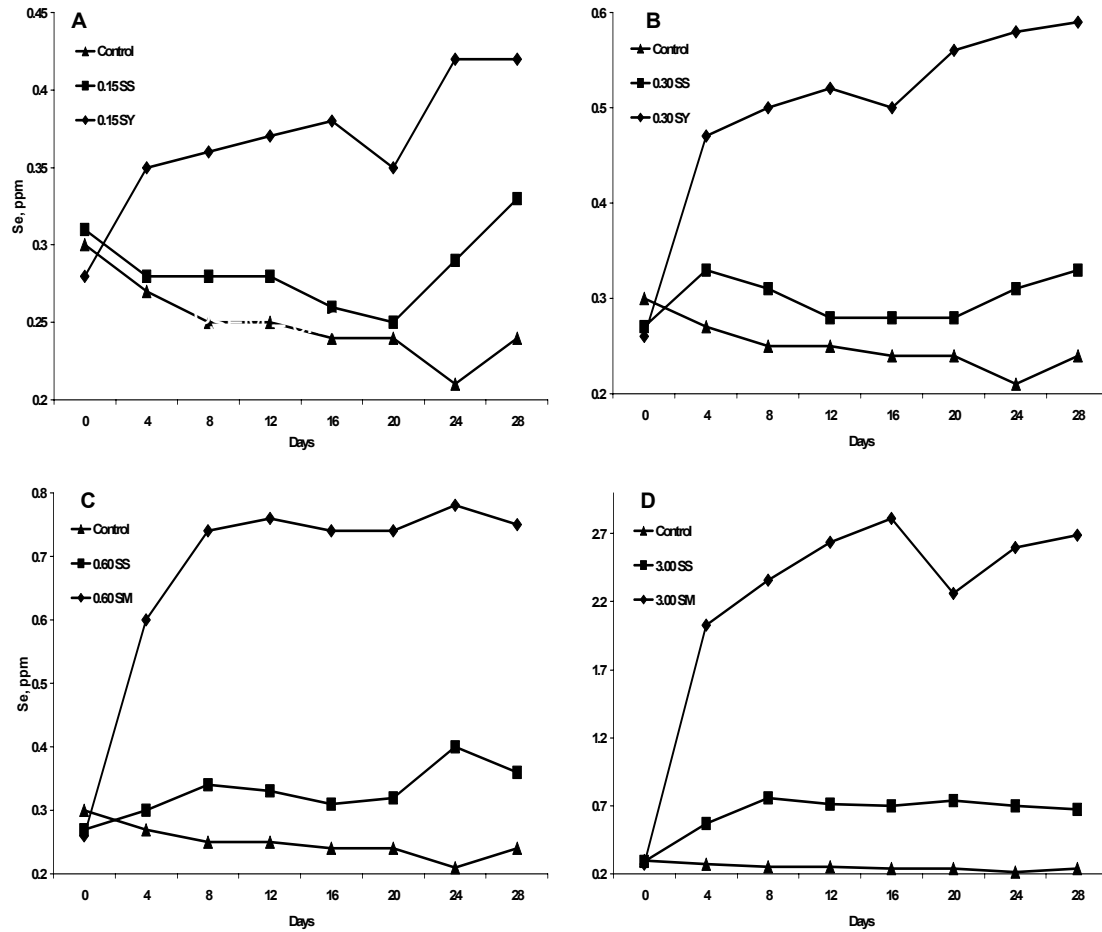


Figure 3.1. Whole-egg selenium concentration in hens fed sodium selenite or selenium-enriched yeast over a 28-d period<sup>1,a,b,c,d,e,f,g,h</sup>

<sup>1</sup>Egg selenium concentration data are means of four (eight hens per replicate) replicates. Two eggs per replicate were collected every 4 d for the duration of the experiment. The experiment lasted 28 d. Data was analyzed using the unstructured repeated measures model of Proc Mixed. The figures are separated within selenium level: A) 0.15 ppm; B) 0.30 ppm; C) 0.60 ppm; and D) 3.00 ppm.

<sup>a</sup>Treatment,  $P < 0.01$ .

<sup>b</sup>Time,  $P < 0.01$ .

<sup>c</sup>Treatment x time,  $P < 0.01$ .

<sup>d</sup>Control vs. selenium-supplemented diets,  $P < 0.01$ .

<sup>e</sup>Source,  $P < 0.01$ .

<sup>f</sup>Source x level linear,  $P < 0.01$ .

<sup>g</sup>Sodium selenite linear,  $P < 0.01$ .

<sup>h</sup>Selenium-enriched yeast linear,  $P < 0.01$ .

actively absorbed and can be directly incorporated into protein, whereas, inorganic Se sources, such as SS, are passively absorbed by the body. Inorganic Se sources, such as SS, are required for selenocysteine synthesis.

The results from our experiment indicate that up to 3 ppm of SS or SY can be supplemented into the diets for laying hens without detrimental effects on daily egg production. Our data also indicate that SY results in a greater deposition of Se in the whole-egg than does SS.

## **CHAPTER 4**

### **COMPARISON OF INORGANIC AND ORGANIC SELENIUM SOURCES FOR BROILERS**

#### **INTRODUCTION**

Selenium was discovered in the flue dust of iron pyrite burners by Berzelius in 1817. It was initially reported to be toxic to animals, but in 1973, Rotruck et al. reported that Se was required for proper function of the antioxidant enzyme, glutathione peroxidase. The glutathione peroxidase enzymes are antioxidant enzymes that destroy free radicals that are produced during normal metabolic activity before any damage can occur. Noguchi et al. (1973) indicated that plasma glutathione peroxidase (GPX-3) activity was directly linked with dietary Se level, and thus, GPX-3 activity is an ideal response variable for evaluating Se supplements. Because Se is a component of GPX and other enzymes, it is a dietary essential nutrient for broilers (NRC, 1994).

The Se requirement for broilers throughout the growth period is 0.15 ppm (NRC, 1994), and this requirement often can be met by natural feedstuffs in the diet. However, due to the considerable regional variation in Se content of natural feedstuffs, it is common practice in the U.S. to supplement broiler diets with Se. The maximum allowable level of Se supplementation is 0.30 ppm (AAFCO, 2003). The Se supplement that primarily has been used in animal diets is the inorganic form, sodium selenite ( $\text{Na}_2\text{SeO}_3$ ; SS). Recently however, there has been interest in the use of organic forms of Se, such as selenocysteine, selenomethionine (SM), or Se-enriched yeast (SY), as supplemental sources of Se. Therefore, the objectives of

this experiment were to compare inorganic with organic sources of Se on growth performance, carcass traits, Se concentration in the breast and plasma, and GPX-3 activity in broilers.

## **MATERIALS AND METHODS**

### **General**

An experiment was conducted to compare the effects of organic with inorganic Se on growth performance, carcass traits, plasma and breast muscle Se concentrations, and GPX-3 activity in commercial broilers in 49-d growth trial typical of the industry. The methods used in this experiment were approved by the Louisiana State University Agricultural Center Animal Care and Use Committee.

Broilers (Ross x Ross) were obtained from a commercial hatchery on d 0 posthatching. They were sexed on d 0 and within sex, randomly allotted to three dietary treatments. Their average initial and final BW were 47 and 2,684 g, respectively. Each treatment was replicated seven times with four replicates of 50 male broilers per replicate and three replicates of 55 female broilers per replicate using a total of 1,095 broilers. The three treatments were: a corn-soybean meal (C-SBM) basal diet with no supplemental Se or this diet supplemented with 0.30 ppm Se from SS, or SY<sup>1</sup>. The SS and SY supplements contained 600 and 2,000 ppm of Se, respectively, and these values were used to determine inclusion levels into the C-SBM diet.

A three-phase feeding program similar to industry recommendations was used. The starting phase was fed from 0 to 17 d, the growing phase from 17 to 35 d, and the finishing phase from 35 to 49 d. The diets were formulated to provide 1.28%

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<sup>1</sup> Sel-Plex, Alltech, Inc. Nicholasville, KY

total Lys and 3,142 kcal/kg of ME for the starting phase, 1.17% total Lys and 3,194 kcal/kg of ME for the growing phase, and 0.92% total Lys and 3,237 kcal/kg of ME for the finishing phase (Table 4.1). The starting phase diets were provided as crumbles and the growing and finishing phase diets were provided as pellets. Feed and water were provided *ad libitum* throughout the experiment. The trace mineral premix used did not contain supplemental Se; otherwise all nutrients met or exceeded the nutrient requirements of broilers (NRC, 1994).

The broilers were housed in a one tunnel-ventilated house with concrete floor pens covered with 14 cm of wood shavings at the Louisiana State University Agricultural Center Poultry Farm. The house contained 30 1.52 x 3.05-m pens. Water was provided to the broilers by an automatic nipple waterer with nine nipples, and the feed was provided via a feed tray from 0 to 7 d and then by two hanging tube feeders (43.2 cm diameter each) for the remainder of the experiment. The houses were maintained at a temperature between 29.4 and 32.2°C for the first week, and then the temperature was decreased 2.8°C every week until the house temperature was between 23.9 and 26.7°C. One infrared brooding lamp per pen was used for the first week. The lighting was via incandescent lights and was similar to commercial conditions; 24:0 L:D from 0- to 4-d posthatching, 10:14 L:D from 5- to 10-d posthatching, 12:12 L:D from 11- to 18-d posthatching, and 16:8 L:D from 19- to 49-d posthatching. The light intensity was 1.0 to 1.3 foot candles from 0- to 10-d posthatching and 0.2 to 0.3 foot candles from 11- to 49-d posthatching.

Table 4.1. Composition of basal diets, as-fed basis<sup>1</sup>

Ingredient	Starter	Grower	Finisher
	control	control	control
Corn	56.34	58.85	69.65
Soybean meal (47.5% CP)	31.57	28.99	19.59
Pork meat and bone meal	5.00	5.00	5.00
Tallow	4.30	4.69	3.67
Limestone	0.13	--	--
Monocalcium phosphate	1.65	1.57	1.29
Salt	0.30	0.30	0.30
DL-Methionine	0.27	0.22	0.17
Trace minerals <sup>2</sup>	0.25	0.25	0.25
Choline chloride	0.03	--	--
Vitamins <sup>3</sup>	0.05	0.05	0.05
Lysine•HCl	0.05	--	--
Biocox 60 <sup>4</sup>	0.05	0.05	--
Ethoxyquin	0.03	0.03	0.03
Calculated composition			
ME, kcal/kg	3,142	3,194	3,237
Crude fat, %	2.91	2.98	3.29
Crude protein, %	22.74	21.65	18.07
Lysine, %	1.28	1.17	0.92
TSAA, %	0.98	0.90	0.76
Calcium, %	0.85	0.78	0.72
Available phosphorous, %	0.46	0.44	0.37
Selenium, ppm	0.12	0.12	0.11

<sup>1</sup>One percent of the basal premix was mixed with the dietary additives for mixing of dietary treatments in the starter phase, while 0.5% of the basal premix was used in the grower and finisher phases. Feed was provided as crumbles during the starter phase and as pellets during the grower and finisher phases.

<sup>2</sup>Provided per kilogram of diet: copper (copper sulfate•5H<sub>2</sub>O), 10 mg; iodine (potassium iodate), 1.4 mg; iron (ferrous sulfate•7H<sub>2</sub>O), 40 mg; manganese (manganese sulfate •H<sub>2</sub>O), 120 mg; and zinc (zinc sulfate•7H<sub>2</sub>O), 100 mg with calcium carbonate as the carrier.

(table 4.1 continued)

<sup>3</sup>Provided per kilogram of diet: vitamin A, 8,000 IU; vitamin D<sub>3</sub>, 3,000 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B<sub>12</sub>, 0.02 mg; biotin, 0.1 mg; folacin, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; and thiamin, 3 mg.

<sup>4</sup>Bio-Cox 60 provided 132.28 grams of salinomycin sodium per kilogram of premix.

At each dietary phase change and at the conclusion of the experiment, all broilers and feed were weighed for calculation of average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (gain:feed). After weighing on d-49 posthatching, six broilers per replicate were randomly selected for processing for carcass trait measurements. On d 50, the broilers were weighed and processed at the LSU Agricultural Center Poultry Abattoir in commercial-type conditions. After scalding, plucking, and evisceration, the broilers were weighed (eviscerated weight) and then placed in an aerated chill tank to allow the carcasses to chill to approximately 7°C. After chilling, the carcasses were drained of any excess surface water for 15 min, and then weighed (chill weight) for calculation of moisture gain due to chill. The whole breast then was removed from each carcass, weighed, and placed on a meat tray with two absorbent pads. The tray containing the breast and wingband of the individual broiler was heat sealed and placed in a cooler (4 to 6°C). After 24 h, the trays were removed from the cooler, and each breast was blotted dry with a paper towel and weighed for calculation of moisture loss. The six broilers that were used for processing also were used for plasma and breast tissue Se concentrations and GPX-3 analysis.

### **Diets, Plasma, and Tissue Selenium Analysis**

The blood from the individual broilers was pooled by pen, placed into 7-mL trace mineral-free tubes with 100 u.s.p. units of sodium heparin<sup>2</sup>, and then centrifuged for 15 min at 3,000 x g at 4°C. After centrifugation, the fresh plasma was collected and analyzed for GPX-3 activity as described by Beutler (1984). The breasts, diets, and plasma samples were analyzed for Se by the methods of Brown

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<sup>2</sup> Vacutainer, BD Vacutainer, Franklin Lakes, NJ

and Watkinson (1977) using a semi-automated fluorimeter<sup>3</sup> at the Oregon State University Forage Analytical Service. All samples were wet digested in nitric and perchloric acids. The Se analyses of the diets are presented in Table 4.2.

Table 4.2. Comparison of calculated selenium levels with analyzed selenium levels in the diets<sup>1</sup>

Treatment	Calculated Se, ppm <sup>2</sup>	Analyzed starter Se, ppm	Analyzed grower Se, ppm	Analyzed finisher Se, ppm
Control	0.00	0.18	0.18	0.15
Control + 0.30 ppm SS	0.30	0.33	0.28	0.31
Control + 0.30 ppm SY	0.30	0.36	0.28	0.33

<sup>1</sup>SS = sodium selenite; and SY = Se-enriched yeast.

<sup>2</sup> Calculated from addition from the Se premix, and not from the natural feedstuffs.

### Statistical Analysis

All data were analyzed by analysis of variance procedures (Steel and Torrie, 1980) appropriate for a completely randomized design by the GLM procedure of SAS<sup>®</sup> (1995). The replicate pen of broilers served as the experimental unit for all data. The model for all response variables contained treatment as the main effect and sex as a covariate, and sex was significant in the model ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

Daily gain, ADFI, gain:feed, and mortality were not affected ( $P > 0.05$ ) by Se source or level of supplementation in any period of growth or in the overall data (Table 4.3). Similarly, carcass traits were not affected ( $P > 0.05$ ) by Se source or level of supplementation (Table 4.4). Breast muscle and plasma Se concentrations

<sup>3</sup> Model 300 with 321 fluorimeter and 303A selenium cartridge, Astoria-Pacific International, Clackamas, OR

were increased ( $P < 0.05$ ) in broilers fed SY but not in those fed SS. Plasma GPX-3 activity was not affected ( $P > 0.05$ ) by Se source or level of supplementation.

Our overall growth performance results are in general agreement with the results of Miller et al. (1972), Edens et al. (2001), and Spears et al. (2003). Miller et al. (1972) reported no difference in gain or feed intake of broilers fed various levels (0 to 0.5 ppm) of Se from SS or SM, while Edens et al. (2001) reported no differences in BW or feed efficiency when broilers were fed diets containing 0.20 ppm Se from SS or SY. Spears et al. (2003) also reported no difference in gain or feed efficiency of broilers fed diets containing 0, 0.05, or 0.15 ppm Se from SS or SM. However, our results do not agree with Cantor et al. (1982), who reported that BW and feed intake were increased in 28-d old turkey poults fed either SS or SM (0.04 to 0.12 ppm Se) compared with those fed a basal diet. Our results for ADFI also do not agree with those of Bunk and Combs (1980), who reported that SS or SM increased feed intake in chicks after oral administration compared with chicks receiving a water-sham. They also reported an increase in gain of chicks fed a diet containing 0.15 ppm SS compared with those fed a basal diet. The differences in their results and ours may be due to the fact that their chicks were Se- and vitamin E-depleted at hatch, whereas our broilers were not deficient in any nutrient at the time of hatch. We observed no difference in mortality due to Se supplementation, which is in agreement with the results of Cantor et al. (1975) and Edens et al. (2001).

Table 4.3. Effect of selenium source on growth performance<sup>1,a</sup>

Item	Control	SS	SY	PSEM
d-0 BW, g	47.19	47.05	47.13	0.22
<i>Starter phase (0 to 17 d posthatching)</i>				
d-17 BW, g	480	484	480	6
ADG, g	26.80	27.02	26.92	0.41
ADFI, g	36.49	35.40	35.72	0.44
Gain:feed, g:g	0.734	0.761	0.753	0.009
Mortality, % <sup>2</sup>	1.95	3.35	1.35	0.24
<i>Grower phase (17 to 35 d posthatching)</i>				
d-35 BW, g	1,830	1,851	1,833	13
ADG, g	70.94	71.26	70.94	0.68
ADFI, g	119.13	118.55	118.24	0.71
Gain:feed, g:g	0.595	0.600	0.600	0.004
Mortality, % <sup>2</sup>	1.54	0.00	0.29	0.18
<i>Finisher phase (35 to 49 d posthatching)</i>				
d-49 BW, g	2,713	2,696	2,684	61
ADG, g	61.14	56.91	56.27	3.77
ADFI, g	159.08	156.18	156.77	4.12
Gain:feed, g:g	0.382	0.361	0.358	0.015
Mortality, % <sup>2</sup>	6.97	6.62	7.10	0.33
<i>Overall (0 to 49 d posthatching)</i>				
ADG, g	53.60	52.46	52.22	1.13
ADFI, g	103.02	100.99	101.18	1.23
Gain:feed, g:g	0.520	0.519	0.516	0.005
Mortality, % <sup>2</sup>	9.40	6.55	7.53	0.38

<sup>1</sup>Data for all treatments are the means of four male and three female replicates. There were 50 male or 55 female broilers per replicate. Average initial and final body weight were 47 and 2,684 g, respectively, and the growth trial lasted 49 d. All growth performance data have been adjusted for mortality. ADG = average daily

(table 4.3 continued)

gain; ADFI = average daily feed intake; SS = sodium selenite; SY = selenium-enriched yeast.

<sup>2</sup>Mortality was analyzed using the square-root transformation ( $\sqrt{y+0.5}$ ). Treatment means are actual means from original data.

<sup>a</sup>There were no differences between treatments,  $P > 0.05$ .

Table 4.4. Effect of selenium source on carcass traits, plasma and breast tissue selenium concentrations, and plasma glutathione peroxidase concentrations of 49 d-old broilers<sup>1</sup>

Item	Control	SS	SY	PSEM
Live weight, g	2,585	2,615	2,635	77
Eviscerated weight, g	1,936	1,964	1,988	55
Chill weight, g	1,994	2,016	2,036	54
Carcass yield, % <sup>2</sup>	75.07	75.00	75.05	0.50
Moisture gain due to chill, % <sup>3</sup>	3.03	2.74	2.47	0.37
Breast weight, g	350.92	346.59	360.52	15.28
24-h breast weight, g	340.52	336.35	350.69	15.00
24-h moisture loss, % <sup>4</sup>	3.05	3.03	2.82	0.20
Breast weight PLW	13.56	13.22	13.68	0.29
Breast weight PCW	17.57	17.15	17.71	0.36
Muscle Se concentration				
Dry matter basis, ppm	0.472 <sup>a</sup>	0.545 <sup>a</sup>	1.170 <sup>b</sup>	0.033
Plasma Se concentration, ppm	0.130 <sup>a</sup>	0.137 <sup>a</sup>	0.160 <sup>b</sup>	0.006
Plasma glutathione peroxidase				
moles of NADPH/min/g protein	5.41	5.74	5.31	0.22

<sup>1</sup>Data for all treatments are the means of four male and three female replicates of six broilers per replicate. There were 50 male or 55 female broilers per replicate. Average initial and final body weight were 47 and 2,684 g, respectively, and the growth trial lasted 49 d. Birds were processed on d 50 after a 12-h fast. PCW = percentage of chill weight; PLW = percentage of live weight; SS = sodium selenite; SY = selenium-enriched yeast.

<sup>2</sup>Carcass yield calculated as eviscerated weight divided by live weight times 100.

<sup>3</sup>Moisture gain calculated as chill weight minus eviscerated weight divided by eviscerated weight times 100.

<sup>4</sup>Moisture loss calculated as 24-h breast weight minus initial breast weight divided by initial breast weight times 100.

<sup>a,b</sup>Treatment means with different superscripts differ  $P < 0.05$ .

We were not able to find any other research that reports the effects of Se on carcass traits of broilers. Broilers fed SY had increased breast and plasma Se levels relative to those fed the control diet or SS. There are several reports comparing tissue or plasma levels of Se in broilers or turkey poult fed diets supplemented with SS or SM, and according to Beilstein and Whanger (1986), the predominant form of Se in SY is SM. Our breast Se results agree with Cantor et al. (1982), who reported increased breast Se levels in turkey poult fed SM compared with those fed a control or SS diet with no differences between the poult fed the control or SS diet. Our results also agree with those of Spears et al. (2003), who reported that broilers fed 0.15 ppm SM had increased breast Se levels than those fed SS. However, they do not agree with the results of Shan and Davis (1994), who reported that broilers fed SS had increased breast Se concentrations than those fed a control diet. Our plasma Se results agree with Cantor et al. (1975), Cantor et al. (1982), Echevarria et al. (1988), and Spears et al. (2003), who all reported that Se supplementation increased plasma Se levels in either turkey poult or broilers.

Sunde (1997) reported that SM can be incorporated into protein at a rate similar to Met, because Se and S have very similar atomic properties. The ability of Se to be treated like S, and the ability of SM to replace Met so that it can be incorporated into protein when metabolized would explain the increase in breast Se in broilers fed SY. The remaining fraction of SY that is not SM should be metabolized in a similar fashion as SS and incorporated into plasma and other selenoproteins.

There were no differences in GPX-3 activity due to Se source or level of supplementation, which is in agreement with Cantor et al. (1975). However, Cantor et al. (1982), Hassan et al. (1988), and Spears et al. (2003) reported that Se supplementation increased GPX-3 activity compared with birds fed unsupplemented diets. Spears et al. (2003) indicated that GPX-3 activity was higher in broilers fed SM compared with SS in one experiment but not in another.

Based on the proposed metabolic pathways for Se (Sunde, 1997), we would not expect any increase in GPX-3 activity from an organic Se source, such as SY. This is because Se, regardless of form, must be converted to selenocysteine before it can be incorporated into the GPX-3 enzyme (Forstrom et al., 1978). Sunde and Hoekstra (1980) reported that inorganic SS was efficiently metabolized into selenocysteine. However, Henry and Ammerman (1995) indicated that SM is converted into selenocysteine at a lower rate of efficiency. Because SS is efficiently metabolized, we would expect that GPX-3 activity would be higher in broilers fed SS compared with those fed an organic source.

Our dietary analysis indicates that we were not able to incorporate the Se supplements at the desired levels into the diets. Although the NRC (1994) indicates a Se requirement of 0.15 ppm, we were attempting to compare these sources at an inclusion level of 0.30 ppm in addition to the Se in the natural feedstuffs of our control diet, because that is the maximum allowed level in the diet (FDA, 2000). According to the analysis, we were able to add approximately 0.15 ppm from each source, which is one-half of our expected inclusion of 0.30 ppm. This lower inclusion level could be due to the fact that we used calculated values for each Se source to

determine our inclusion levels. However, we do not feel the products have so much variation in Se content to reduce the dietary inclusion levels to that degree. It is possible that there was a problem with the selenium analyses, especially considering that the diets were roughly one-half of their expected value in each growth phase. Overall, we do not feel that this was a detriment to our trial but the inconsistencies in the diet analysis need further attention.

The results from this experiment indicate that an organic source of Se, such as SY, can increase tissue Se concentration, while maintaining GPX-3 activity compared with an inorganic source, such as SS.

## CHAPTER 5

### CHANGES IN GLUTATHIONE PEROXIDASE AND TISSUE SELENIUM CONCENTRATIONS OF BROILERS AFTER CONSUMING A DIET ADEQUATE IN SELENIUM

#### INTRODUCTION

Animals, including poultry, produce free radicals as by-products of metabolism that could potentially damage or destroy biological molecules in cells. Superoxide dismutase, catalase, and glutathione peroxidase are a few of the antioxidant enzymes that destroy these free radicals before damage occurs (Öztürk-Ürek et al., 2001). Mills discovered glutathione peroxidase (GSH-Px) in 1957, and then in 1973, Rotruck et al. identified Se as an integral part of the enzyme. Since that time, six GSH-Px enzymes have been identified, four of which require Se in the form of selenocysteine for optimal activity (Sunde, 1997). The primary functions of the GSH-Px enzymes are to detoxify hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and to convert lipid hydroperoxides to non-toxic alcohols (Jenkinson et al., 1982). Of the four Se-dependent GSH-Px enzymes, the activity of the plasma GSH-Px (GPX-3) has been directly linked with dietary Se level (Noguchi et al., 1973).

Selenium is essential in poultry diets in order to protect from exudative diathesis and pancreatic fibrosis, as well as maintain GSH-Px activity (Levander, 1986; NRC, 1994). Historically, diets often have been supplemented with an inorganic form of Se, sodium selenite (SS). However, previous research in our lab suggested that an organic form of Se, Se-enriched yeast (SY), was deposited into the breast muscle of broilers at a much greater rate than SS (chapter 4). However, this research indicated that there was very little difference in growth performance or

GPX-3 concentration in broilers fed diets supplemented with 0.30 ppm of SS or SY. These results caused us to question whether the Se in the breast muscle of broilers fed SY would be of benefit to the broiler during periods of Se inadequacy, with particular attention paid to GPX-3. Therefore, the objectives of these experiments (EXP) were to validate a Se-deficient diet that would maintain growth performance similar to a typical corn-soybean meal diet (C-SBM), and then to determine if the Se that has been deposited into the breast during a Se-loading period could be utilized for pGPX3 activity during a Se-depletion period.

## **MATERIALS AND METHODS**

### **General**

Three EXP were conducted to develop a Se-deficient diet, and then to compare GPX-3 and plasma and tissue Se concentrations in broilers fed a Se-deficient diet after they had been fed diets supplemented with either SS or SY. The Se sources compared in these EXP were SS and SY<sup>1</sup>, which contained 600 and 2,000 ppm of Se, respectively. All diets in these EXP were formulated to provide 1.26% total Lys and 3,200 kilocalories of ME per kilogram of diet, and all nutrients, except Se, met or exceeded the nutrient requirements (NRC, 1994) for 0 to 21 d-old broilers. Torula yeast<sup>2</sup> (TY) was analyzed for amino acids and Se before the start of these EXP (Table 5.1), and it was chosen as a feedstuff because of its low Se content. The amino acid and Se analysis of TY was used in diet formulation. The amino acid concentrations for C and SBM used in diet formulation were from NRC (1994). All diets were fed in mash form, and the feed and water were provided *ad libitum*

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<sup>1</sup> Sel-Plex, Alltech, Inc. Nicholasville, KY

<sup>2</sup> Torutein 10, Provesta Flavor Ingredients, Hutchinson, MN

throughout all EXP. The Louisiana State University Agricultural Center Animal Care and Use Committee approved the methods used in these EXP.

Table 5.1. Analyzed total amino acid and selenium composition of torula yeast, as-fed basis<sup>1</sup>

Nutrient	Torula yeast
Crude protein, %	51.17
Arginine, %	2.99
Cysteine, %	0.52
Histidine, %	1.02
Isoleucine, %	2.26
Leucine, %	3.41
Lysine, %	3.39
Methionine, %	0.64
Phenylalanine, %	2.00
Threonine, %	2.28
Tryptophan, %	0.59
Valine, %	2.72
Selenium, ppm	0.008

<sup>1</sup>Torutein 10, Provesta Flavor Ingredients, Hutchinson, MN.

## Experiments 1 and 2

The purpose of EXP 1 and 2 were to validate a diet deficient in Se that would provide similar growth performance to a typical C-SBM diet. For each EXP, 288 Ross x Ross female broilers were obtained from a commercial hatchery on d 0 posthatching, and then they were weighed, wingbanded, and allotted to dietary treatment. The broilers were then placed in environmentally controlled brooder batteries with raised wire floors and continuous fluorescent lighting. The duration of EXP 1 and 2 was 21 and 20 d, respectively. The initial BW of the broilers for EXP 1

and 2 were 35 and 39 g, and the final BW of the broilers for EXP 1 and 2 were 459 and 624 g, respectively.

In EXP 1, a C-SBM diet, a C-TY diet, a cornstarch-dextrose-TY diet (CS-D-TY), and a CS-D-SBM + 20% TY diet were fed with or without 0.30 ppm of supplemental Se from SS (Table 5.2). The calculated (NRC, 1994) Se levels in the unsupplemented C-SBM, C-TY, CS-D-TY, and CS-D-SBM diets were 0.05, 0.02, 0.01, and 0.02 ppm, respectively, which came from the Se in the feedstuffs. Each treatment was replicated six times with six broilers per replicate.

We were not able to validate a Se-deficient diet in EXP 1, but we were able to narrow our diet type to a CS-D-SBM-TY diet. In EXP 2, a C-SBM diet, a CS-D-SBM + 10% TY, and a CS-D-SBM + 15% TY were fed with or without 0.30 ppm supplemental Se from SS. The calculated (NRC, 1994) Se levels in the C-SBM, CS-D-SBM + 10% TY, and CS-D-SBM + 15% TY diets were 0.05, 0.03, and 0.03 ppm, respectively, which came from the Se in the feedstuffs. Each treatment was replicated eight times with six broilers per replicate.

At the end of EXP 1 and 2, the broilers and feeders were weighed for determination of average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (gain:feed). Blood also was collected from all broilers in EXP 2 and pooled by replicate pen for collection of plasma and determination of GPX-3 activity.

### **Experiment 3**

The purpose of EXP 3 was to determine if broilers fed diets supplemented with 0.30 ppm of SS or SY during a 10-d loading period would maintain GPX-3 activity similarly after the broilers were fed a Se-deficient diet. Three hundred and

Table 5.2. Basal diet composition, as-fed basis<sup>1</sup>

Ingredient	C-SBM	C-TY	CS-D-TY	CS-D-SBM	CS-D-SBM	CS-D-SBM
				+ 10% TY	+ 15% TY	+ 20% TY
Corn	52.75	57.06	-	-	-	-
Cornstarch	-	-	27.37	25.53	26.18	26.83
Dextrose	-	-	26.50	25.00	25.00	25.00
Soybean meal (47.5%CP)	37.93	-	-	31.11	25.39	19.66
Torula yeast <sup>2</sup>	-	32.79	37.17	10.00	15.00	20.00
Oil	5.11	5.82	4.34	3.86	3.92	3.97
Monocalcium phosphate	1.54	1.93	2.14	1.82	1.88	1.94
Limestone	1.54	1.16	1.03	1.36	1.30	1.24
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix <sup>3</sup>	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix <sup>4</sup>	0.05	0.05	0.05	0.05	0.05	0.05
DL-methionine	0.32	0.45	0.61	0.49	0.51	0.53
Choline chloride	-	-	0.04	0.03	0.03	0.04
Calculated Composition						
ME, kcal/kg	3,200	3,200	3,200	3,200	3,200	3,200
ME:Lys	2,540	2,540	2,540	2,540	2,540	2,540
Crude protein, %	22.69	20.33	17.60	19.71	19.32	18.93
Lysine, %	1.26	1.26	1.26	1.26	1.26	1.26
TSAA, %	1.03	1.03	1.03	1.03	1.03	1.03

(table 5.2 continued)

Calcium, %	1.00	1.00	1.00	1.00	1.00	1.00
Avail. Phosphorous, %	0.45	0.45	0.45	0.45	0.45	0.45
Selenium, ppm	0.054	0.024	0.007	0.033	0.028	0.024

<sup>1</sup>The basal diets were supplemented with 0 or 0.30 ppm sodium selenite or Se-enriched yeast in each EXP. C = corn; CS = cornstarch; D = dextrose; SBM = soybean meal; and TY = torula yeast.

<sup>2</sup>Torutein 10, Provesta Flavor Ingredients, Hutchinson, MN.

<sup>3</sup>Provided per kilogram of diet: copper (copper sulfate•5H<sub>2</sub>O), 10 mg; iodine (potassium iodate), 1.4 mg; iron (ferrous sulfate•7H<sub>2</sub>O), 40 mg; manganese (manganese sulfate •H<sub>2</sub>O), 120 mg; and zinc (zinc sulfate•7H<sub>2</sub>O), 100 mg with calcium carbonate as the carrier.

<sup>4</sup>Provided per kilogram per diet: vitamin A (vitamin A palmitate), 8,000 IU; vitamin D<sub>3</sub>, 3,000 IU; vitamin E (vitamin E acetate), 25 IU; menadione (menadione sodium bisulfite), 1.5 mg; vitamin B<sub>12</sub>, 0.02 mg; biotin (d-biotin), 0.1 mg; folacin (folic acid), 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; and thiamin (thiamin•HCL), 3 mg.

sixty female Ross x Ross broilers were obtained from a commercial hatchery on d 0 posthatching, and they were allotted to treatments and penned as previously described. The EXP lasted from d 0 to d 22 posthatching. The initial and final BW of the broilers were 36 and 778 g, respectively.

From d 0 to d 10 posthatching, the broilers were fed a C-SBM diet with no supplemental Se or 0.30 ppm of Se from SS or SY. The C-SBM diet was the same as used in EXP 2 (Table 5.2). Each treatment was replicated eight times with 15 broilers per replicate. On d 0 posthatching, ten broilers were randomly selected for determination of baseline GPX-3 and breast, liver, and plasma Se concentrations. Blood was collected and pooled, and GPX-3 was determined on fresh plasma as described below. The left breast and one lobe of the liver from each broiler also were collected and pooled.

On d 10 posthatching, all of the feeders were removed and weighed for calculation of ADFI. The broilers were then placed on the CS-D-SBM + 10% TY diet, which contained 0.03 ppm of Se on a calculated basis, for the remainder of the trial. On d 10, 13, 16, 19, and 22, three broilers per replicate were randomly selected and weighed. Blood, breast, and liver samples were collected from each broiler as described above and pooled by replicate pen for determination of GPX-3 activity and tissue Se concentrations.

### **Diets, Plasma, and Tissue Selenium Analysis**

The amino acid composition of TY, with the exception of the Met, Cys, and Trp, was determined after acid hydrolysis (AOAC, 2003). The Met and Cys composition of TY was determined after performic acid oxidation followed by acid

hydrolysis, and the Trp concentration of TY was determined after alkaline hydrolysis (AOAC, 2003).

The blood from the broilers in EXP 2 and 3 were collected via cardiac puncture and pooled by replicate pen. The blood was placed in 7-mL trace mineral-free tubes with 100 u.s.p. units of sodium heparin<sup>3</sup>. After collection, the blood samples were centrifuged for 15 min at 3,000 x g at 4°C. The plasma was collected and GPX-3 activity was determined on fresh plasma as described by Beutler (1984).

The diets and pooled plasma samples from all EXP and the pooled breast and liver tissue samples from EXP 3 were analyzed for Se by the methods of Brown and Watkinson (1977) using a semi-automated fluorimeter<sup>4</sup> at the Oregon State University Forage Analytical Service Laboratory (Tables 5.3, 5.5, 5.6, and 5.7). All samples were wet digested in nitric and perchloric acids.

### **Statistical Analysis**

Data in all EXP were analyzed by analysis of variance procedures (Steel and Torrie, 1980) appropriate for completely randomized designs by the GLM procedure of SAS<sup>®</sup> (1995). The GPX-3 area-under-the response curve was determined using trapezoidal geometry. All treatment effects noted are significant at  $P < 0.05$ .

## **RESULTS**

### **Experiments 1 and 2**

In EXP 1, broilers fed the C-TY diets, CS-D-TY diets, or the CS-D-SBM + 20% TY diets with or without supplemental Se had decreased ADG, ADFI, and gain:feed compared with broilers fed the C-SBM diets (Table 5.4). However, the

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<sup>3</sup> Vacutainer, BD Vacutainer, Franklin Lakes, NJ.

<sup>4</sup> Model 300 with 321 fluorimeter and 303A selenium cartridge, Astoria-Pacific International, Clackamas, OR.

Table 5.3. Analysis of diets for selenium concentration, as-fed basis<sup>1</sup>

Treatment	Calculated Se, ppm	Analyzed Se, ppm
<i>Experiment 1</i>		
C-SBM	0.054	0.169
C-SBM + 0.30 ppm SS	0.354	0.348
C-TY	0.024	0.046
C-TY + 0.30 ppm SS	0.324	0.225
CS-D-TY	0.007	0.011
CS-D-TY + 0.30 ppm SS	0.307	0.203
CS-D-SBM + 20% TY	0.024	0.028
CS-D-SBM + 20% TY + 0.30 ppm SS	0.324	0.244
<i>Experiment 2</i>		
C-SBM	0.054	0.118
C-SBM + 0.30 ppm SS	0.354	0.370
CS-D-SBM + 10% TY	0.033	0.042
CS-D-SBM + 10% TY + 0.30 ppm SS	0.333	0.269
CS-D-SBM + 15% TY	0.028	0.059
CS-D-SBM + 15% TY 0.30 ppm SS	0.328	0.268
<i>Experiment 3</i>		
C-SBM	0.054	0.067
C-SBM + 0.30 ppm SS	0.354	0.251
C-SBM + 0.30 ppm SY	0.354	0.286
CS-D-SBM + 10% TY	0.033	0.056

<sup>1</sup>C = corn; CS = cornstarch; D = dextrose; SBM = soybean meal; SS = sodium selenite; SY = Se-enriched yeast; and TY = torula yeast

Table 5.4. Growth performance of chicks for validation of a selenium deficient diet, Experiment 1<sup>1</sup>

Treatment	ADG, g	ADFI, g	G:F, g:g
1. C-SBM	30.44 <sup>a</sup>	38.51 <sup>a</sup>	0.790 <sup>a</sup>
2. 1 + 0.30 ppm SS	32.16 <sup>a</sup>	40.64 <sup>a</sup>	0.792 <sup>a</sup>
3. C-TY diet	16.37 <sup>b</sup>	27.63 <sup>b</sup>	0.604 <sup>b</sup>
4. 3 + 0.30 ppm SS	18.32 <sup>b</sup>	29.65 <sup>b</sup>	0.623 <sup>b</sup>
5. CS-D-TY diet	6.83 <sup>c</sup>	19.71 <sup>c</sup>	0.348 <sup>c</sup>
6. 5 + 0.30 ppm SS	7.62 <sup>c</sup>	20.74 <sup>c</sup>	0.373 <sup>c</sup>
7. CS-D-SBM + 20% TY	26.64 <sup>d</sup>	40.88 <sup>a</sup>	0.651 <sup>b,d</sup>
8. 7 + 0.30 ppm SS	26.81 <sup>d</sup>	38.63 <sup>a</sup>	0.694 <sup>d</sup>
Pooled SEM	0.67	1.15	0.023

<sup>1</sup>Data are means of six replicates of six broilers per replicate. Average initial and final body weight were 35 and 459 g, respectively. The growth trial lasted for 21 d. C = corn; CS = cornstarch; Dex = dextrose; SBM = soybean meal; SS = sodium selenite; and TY = torula yeast;

<sup>a,b,c,d</sup>Means with different superscripts differ, P < 0.05.

broilers fed the CS-D-SBM + 20% TY diets had growth performance more similar to those fed the C-SBM diets and better growth performance than those fed the C-TY or CS-D-TY diets. Based on the results of this EXP, we were able to determine that the CS-D-SBM + 20% TY diet was the type of diet we would need to use in our EXP.

In EXP 2, Se supplementation to the C-SBM diet did not affect ADG, ADFI, gain:feed or GPX-3 levels (Table 5.5). However, plasma Se levels were increased by Se supplementation. Broilers fed the CS-D-SBM + 10% TY diets with or without supplemental Se had similar ADG to those fed the C-SBM diets. However, ADFI was increased and gain:feed was decreased in broilers fed the CS-D-SBM + 10% TY diets compared with those fed the C-SBM diets. Plasma Se and GPX-3 concentration of broilers fed the CS-D-SBM + 10% TY diet without Se supplementation was lower than those fed the same diet with Se supplementation, and Se supplementation to this diet resulted in similar plasma Se and GPX-3 concentrations to those broilers fed the C-SBM diets with or without supplemental Se. The broilers fed the CS-D-SBM + 15% TY without supplemental Se had similar ADG compared with those fed the C-SBM diet without supplemental Se. However, when Se was added to the CS-D-SBM + 15% TY diet, ADG was decreased compared with those fed the C-SBM diets. Plasma Se and GPX-3 concentrations were decreased in broilers fed the CS-D-SBM + 15% TY without Se supplementation compared with those fed the C-SBM diets, but when supplemented with Se, plasma Se and GPX-3 were the same as broilers fed the C-SBM diets with or without supplemental Se. Feed intake and gain:feed were decreased in broilers fed the CS-D-SBM + 15% TY diet with or without supplemental Se compared with

Table 5.5. Growth performance, plasma selenium, and plasma glutathione peroxidase concentrations of chicks for validation of a selenium deficient diet, Experiment 2<sup>1</sup>

Treatment	ADG, g	ADFI, g	G:F, g:g	Plasma	
				GPX-3 <sup>2</sup>	Se, ppm
1. C-SBM	29.99 <sup>a</sup>	38.87 <sup>a,b,d</sup>	0.772 <sup>a</sup>	2.61 <sup>a</sup>	0.117 <sup>a</sup>
2. 1 + 0.30 ppm SS	30.42 <sup>a</sup>	38.38 <sup>b</sup>	0.793 <sup>a</sup>	2.78 <sup>a</sup>	0.129 <sup>b,d</sup>
3. CS-D-SBM + 10% TY	30.62 <sup>a</sup>	43.52 <sup>c,e</sup>	0.704 <sup>b</sup>	0.29 <sup>b</sup>	0.019 <sup>c</sup>
4. 3 + 0.30 ppm SS	29.43 <sup>a,b</sup>	42.08 <sup>c,e</sup>	0.699 <sup>b</sup>	2.48 <sup>a</sup>	0.111 <sup>a</sup>
5. CS-D-SBM + 15% TY	28.75 <sup>a,b</sup>	42.18 <sup>c,e</sup>	0.683 <sup>b,c</sup>	0.74 <sup>c</sup>	0.019 <sup>c</sup>
6. 5 + 0.30 ppm SS	27.85 <sup>b</sup>	41.69 <sup>d,e</sup>	0.669 <sup>c</sup>	2.50 <sup>a</sup>	0.118 <sup>d</sup>
Pooled SEM	0.68	1.02	0.009	0.14	0.004

<sup>1</sup>Data are means of eight replicates of six broilers per replicate. Average initial and final body weight were 35 and 459 g, respectively. The growth trial lasted for 21 d. C = corn; CS = cornstarch; Dex = dextrose; SBM = soybean meal; SS = sodium selenite; and TY = torula yeast.

<sup>2</sup>Glutathione peroxidase (GPX-3) is expressed as moles of NADPH oxidized per min per g of protein.

<sup>a,b,c,d,e</sup>Means with different superscripts differ, P < 0.05.

those fed the C-SBM diets. Based on the results of EXP 2, we felt that the CS-D-SBM + 10% TY met the requirements of validation, which was to provide similar growth performance as a typical C-SBM diet, and that when supplemented with Se resulted in similar plasma Se and GPX-3 as broilers fed the C-SBM diet.

Furthermore, we wanted a diet that would provide essentially no Se to the broilers, and this diet resulted in the lowest plasma GPX-3 concentration. Therefore, the CS-D-SBM + 10% TY diet without Se supplementation was used for the Se depletion period of EXP 3.

### **Experiment 3**

During the period of adequate Se intake (d 0 to 10) and during the overall period (d 0 to 22), there were no differences in final BW, ADG, ADFI, or gain:feed in broilers fed the C-SBM diet with or without supplemental Se (Table 5.6). Similarly, diet did not affect growth performance at any time period (d 10, 13, 16, 19, or 22).

Plasma Se and GPX-3 concentrations were increased in broilers fed the diets supplemented with Se compared with those fed the C-SBM diet without supplemental Se on each sample collection day throughout the EXP (Tables 5.6 and 5.7; Figures 5.1a and 5.1b). The GPX-3 concentration was similar in broilers fed either SS or SY on d 10 and 13, but on d 16, 19, and 22, broilers fed SY had increased GPX-3 concentration compared with those fed SS. However, area under the response curve for GPX-3 concentrations were not affected by Se source. Broilers fed the C-SBM without supplemental Se had lower area under the response curve for GPX-3 concentrations compared with those fed either Se source. Plasma

Table 5.6. Growth performance and plasma glutathione peroxidase concentrations of chicks during selenium loading and depletion periods, Experiment 3<sup>1</sup>

Treatment	C-SBM	SS	SY	PSEM
Day 10 FW, g	230.33	223.12	229.46	5.14
Day 13 FW, g	330.42	330.67	330.00	8.95
Day 16 FW, g	456.33	451.13	455.00	8.64
Day 19 FW, g	615.50	624.21	600.62	11.52
Day 22 FW, g	780.81	777.17	775.33	15.34
<i>Growth performance during period of selenium intake (d 0 to 10)</i>				
ADG, d 0-10, g	19.45	18.71	19.32	0.50
ADFI, d 0-10, g	21.07	20.62	20.89	0.36
Gain:Feed, g:g	0.924	0.906	0.925	0.015
<i>Overall growth performance (d 0 to 22)</i>				
ADG, d 0-22, g	33.86	33.70	33.60	0.69
ADFI, d 0-22, g	46.63	45.66	46.17	0.68
Gain:Feed, g:g	0.727	0.738	0.727	0.011
<i>GPX-3, moles of NADPH oxidized per min per g of protein<sup>2</sup></i>				
Day 0	1.94	1.94	1.94	--
Day 10	0.86 <sup>a</sup>	3.32 <sup>b</sup>	3.42 <sup>b</sup>	0.20
Day 13	0.64 <sup>a</sup>	1.93 <sup>b</sup>	2.27 <sup>b</sup>	0.15
Day 16	0.40 <sup>a</sup>	1.01 <sup>b</sup>	1.31 <sup>c</sup>	0.08
Day 19	0.44 <sup>a</sup>	0.69 <sup>b</sup>	1.01 <sup>c</sup>	0.06
Day 22	0.39 <sup>a</sup>	0.57 <sup>b</sup>	0.73 <sup>c</sup>	0.04
<i>Area under the response curve for GPX-3 concentrations per day<sup>3</sup></i>				
Day 10-13	-0.11 <sup>a</sup>	-0.70 <sup>b</sup>	-0.57 <sup>b</sup>	0.14
Day 13-16	-0.34 <sup>a</sup>	-1.85 <sup>b</sup>	-1.63 <sup>b</sup>	0.24
Day 16-19	-0.44 <sup>a</sup>	-2.47 <sup>b</sup>	-2.26 <sup>b</sup>	0.20

(table 5.6 continued)

Day 19-22	-0.44 <sup>a</sup>	-2.69 <sup>b</sup>	-2.55 <sup>b</sup>	0.19
Day 10-22	-0.33 <sup>a</sup>	-1.92 <sup>b</sup>	-1.75 <sup>b</sup>	0.19

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<sup>1</sup>Data for each time period are means of eight replicates of three broilers per replicate. Average initial body weight was 36 g. The growth trial lasted for 22 d. C-SBM = corn-soybean meal diet; GPX-3 = plasma glutathione peroxidase; SS = sodium selenite; SY = Se-enriched yeast.

<sup>2</sup>Glutathione peroxidase (GPX-3) is expressed as moles of NADPH oxidized per min per g of protein.

<sup>3</sup>Area under the response curve was calculated using trapezoidal geometry.

<sup>a,b,c</sup>Means within a row with different superscripts differ,  $P < 0.05$ .

Table 5.7. Plasma and tissue selenium concentrations of chicks during selenium loading and depletion periods, Experiment 3<sup>1</sup>

Treatment	C-SBM	0.30 ppm SS	0.30 ppm SY	PSEM
<i>Plasma selenium concentrations, ppm</i>				
Day 0	0.158	0.158	0.158	--
Day 10	0.036 <sup>a</sup>	0.112 <sup>b</sup>	0.116 <sup>b</sup>	0.005
Day 13	0.026 <sup>a</sup>	0.062 <sup>b</sup>	0.073 <sup>c</sup>	0.003
Day 16	0.021 <sup>a</sup>	0.038 <sup>b</sup>	0.056 <sup>c</sup>	0.002
Day 19	0.028 <sup>a</sup>	0.039 <sup>b</sup>	0.050 <sup>c</sup>	0.001
Day 22	0.032 <sup>a</sup>	0.038 <sup>b</sup>	0.042 <sup>b</sup>	0.002
<i>Liver selenium concentrations, ppm (DM basis)</i>				
Day 0	2.273	2.273	2.273	--
Day 10	0.781 <sup>a</sup>	1.624 <sup>b</sup>	1.920 <sup>c</sup>	0.027
Day 13	0.525 <sup>a</sup>	0.949 <sup>b</sup>	1.168 <sup>c</sup>	0.032
Day 16	0.511 <sup>a</sup>	0.719 <sup>b</sup>	0.806 <sup>c</sup>	0.023
Day 19	0.393 <sup>a</sup>	0.444 <sup>a,b</sup>	0.489 <sup>b</sup>	0.024
Day 22	0.428 <sup>a</sup>	0.528 <sup>b</sup>	0.565 <sup>b</sup>	0.022
<i>Breast selenium concentrations, ppm (DM basis)</i>				
Day 0	1.12	1.12	1.12	--
Day 10	0.380 <sup>a</sup>	0.480 <sup>b</sup>	1.21 <sup>c</sup>	0.020
Day 13	0.307 <sup>a</sup>	0.343 <sup>b</sup>	0.709 <sup>c</sup>	0.012
Day 16	0.282 <sup>a</sup>	0.327 <sup>b</sup>	0.604 <sup>c</sup>	0.014
Day 19	0.255 <sup>a</sup>	0.289 <sup>b</sup>	0.467 <sup>c</sup>	0.008
Day 22	0.241 <sup>a</sup>	0.249 <sup>a,b</sup>	0.418 <sup>b</sup>	0.014

<sup>1</sup>Data for each time period are means of eight replicates of three broilers per replicate. Average initial body weight was 36 g. The growth trial lasted for 22 d. C-SBM = corn-soybean meal diet; SS = sodium selenite; SY = Se-enriched yeast.

<sup>a,b,c</sup>Means within a row with different superscripts differ, P < 0.05.

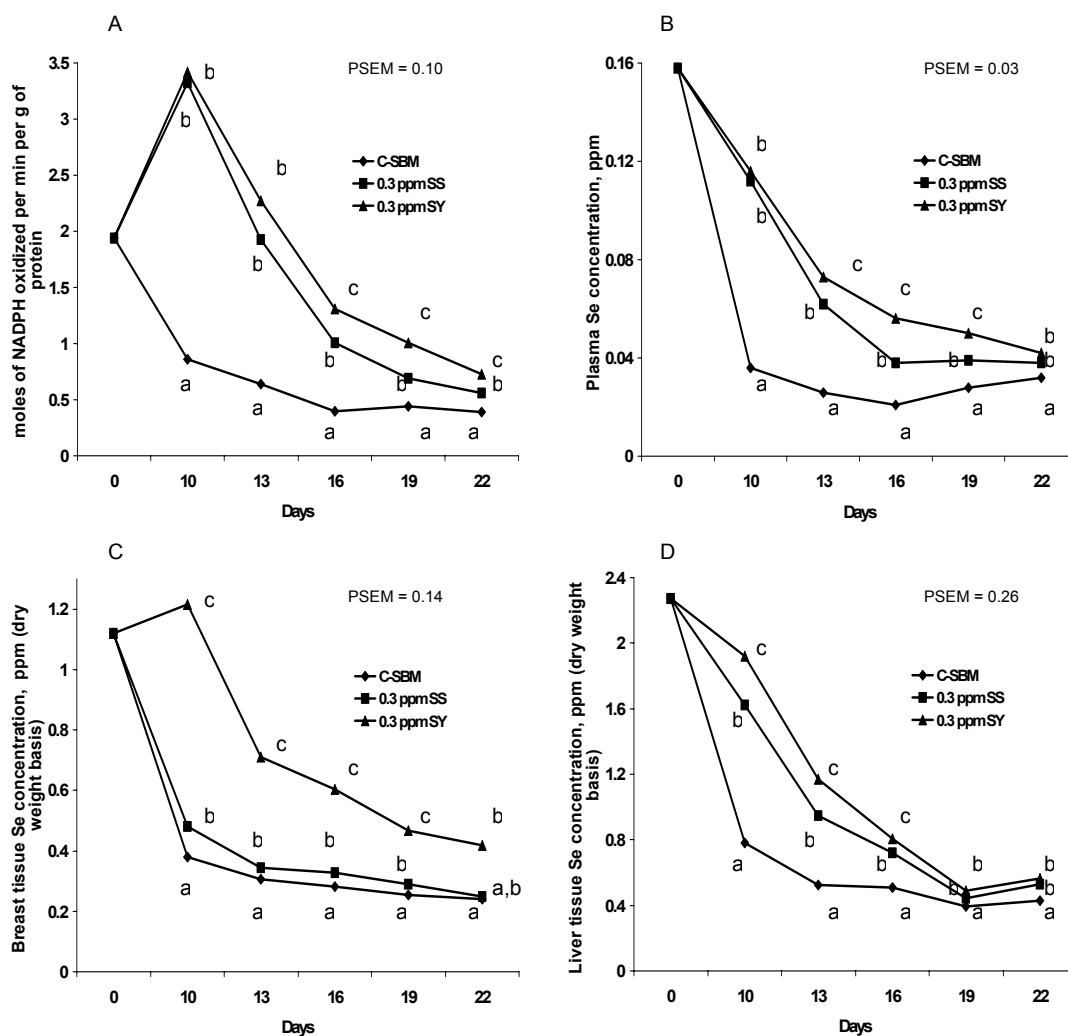


Figure 5.1<sup>1,2</sup>. Plasma glutathione peroxidase activity (A) and selenium concentrations of the plasma (B), breast (C), and liver (D) tissues over time after a 10-d Se-loading period.

<sup>1</sup>Broilers were fed diets supplemented with 0 or 0.30 ppm Se from sodium selenite (SS) or Se-enriched yeast (SY) from d 0 to 10. On d 10, all broilers were placed on a low Se diet for the remainder of the experiment. Data for each time period are means of eight replicates of three broilers per replicate.

<sup>2</sup>Means within day with different superscripts differ,  $P < 0.05$ .

Se concentrations were similar in broilers fed either SS or SY on d 10 and 22, but broilers fed SY had higher plasma Se levels on d 13, 16, and 19 relative to those fed SS.

Breast and liver Se concentrations were higher in broilers fed SS or SY on d 10, 13, and 16 than in those fed the C-SBM diet (Table 5.7; Figures 5.1c and 5.1d). On d 19, broilers fed SY had higher breast and liver Se concentrations than those fed the C-SBM diet. The liver Se level of broilers fed SS was intermediate between SY and the C-SBM, while their breast Se was lower than those fed SY but higher than those fed the C-SBM diet. On d 22, broilers fed either Se source had higher liver Se relative to those fed the C-SBM diet, and those fed SY had higher breast Se than broilers fed the C-SBM diet with those fed SS having an intermediate level of Se in the breast.

## **DISCUSSION**

The first objective of these EXP was to develop and validate a diet deficient in Se that would maintain growth performance similar to a typical C-SBM diet, but this diet would create a Se deficiency in broilers as assessed by GPX-3 when the diet was not supplemented with Se. Plasma GPX-3 activity is closely related to dietary Se concentration according to the results of Noguchi et al. (1973) and Omaye and Tappel (1974). Therefore, this diet would allow us to detect differences in our EXP that would be due to Se supplementation and to allow us to make concise implications regarding the use of low-Se diets in the poultry industry.

Several authors have used Se-deficient diets in previous work for assessing bioavailability of various Se supplements (Thompson and Scott, 1969; Ikumo et al.,

1978; Gabrielsen and Opstvedt, 1980) or for assessing enzyme activity and deficiency signs of Se (Lawrence and Burk, 1976; Fidler et al., 1980; Halpin and Baker, 1984). However, these authors did not report validating their Se-deficient diets, most of which were semi-purified diets containing various levels of TY, against a typical C-SBM diet. Without validation of the experimental diets, it is difficult to interpret the results. If a diet does not result in maximum performance when supplemented with Se, the results may not be applicable or directly comparable to a traditional diet.

Torula yeast is often used to formulate diets that are deficient in Se because it has a low Se content (less than 0.1 ppm) and good amino acid profile. In EXP 1 and 2, we evaluated several different diets with varying levels of TY in an attempt to develop a diet that would result in growth performance similar to broilers fed a C-SBM diet. We feel that our CS-D-SBM + 10% TY diet meets our validation requirements because it resulted in growth performance similar to broilers fed a C-SBM, and broilers fed this diet without Se supplementation were deficient in Se as assessed by GPX-3 activity. Furthermore, when Se was added to this diet, growth performance and GPX-3 remained consistent with that of broilers fed the C-SBM.

When the percentage of TY in the diet was at or above 15%, growth performance was decreased. This response was observed even though the diets were formulated to be nutritionally equivalent. Several others have reported similar decreases in performance from yeast products. Shannon and McNab (1972), van Weerden et al. (1976), Ikumo et al. (1978), and El Boushy and Binnerts (1981) reported that growth performance was decreased in chicks fed diets containing 20%

or more yeast. van Weerden et al. (1976) also reported that pelleting the diets with yeast improved growth performance, which suggests that bulk density could be part of the problem. In EXP 2, the bulk density of the C-SBM, CS-D-SBM + 10% TY, and CS-D-SBM + 15% TY diets were 0.60, 0.58, and 0.51 g/cm<sup>3</sup>, respectively. Also, growth performance was reduced in chicks fed the CS-D-SBM + 15% diet.

On d 10 of EXP 3, which was the last day of Se supplementation, GPX-3 activities in our EXP were similar in broilers fed either SY or SS, and both of these GPX-3 activities were higher than in chicks fed the C-SBM diet with no Se supplementation. These results, which indicate similar levels of GPX-3 activity in the broilers fed SS or SY, agree with those of Cantor et al. (1975, 1982), Shan and Davis (1994), and previous work conducted in our laboratory (unpublished data). Plasma Se levels on d 10 also were similar in broilers fed SS or SY, which agrees with the results of Cantor et al. (1975, 1982). Broilers fed SY had greater liver and breast Se concentrations than those fed SS or those fed the non-supplemented C-SBM diet on d 10. Our breast tissue Se results on d 10 are in general agreement with the results of Cantor et al. (1982), Echevarria et al. (1988a,b), and Spears et al. (2003). Our liver tissue Se results are in partial agreement with Spears et al. (2003), who reported that liver Se level was increased in broilers fed a diet containing SS relative to those fed a diet containing SM, but they were not able to repeat these results in a second EXP. However, our liver Se results do not agree with Cantor et al. (1982), who reported no difference in liver tissue Se when turkey poults were fed either SS or SM.

The second objective of these EXP was to determine if the Se that was deposited in tissue during a period of Se intake would be utilized for GPX-3 activity by the broiler during a period of Se deficiency. Previous research in our laboratory (unpublished data) indicated that broilers fed SY had higher breast Se concentrations than those fed SS. This increase in breast Se concentration may be because the predominant form of Se in SY is SM (Beilstein and Whanger, 1985), which is readily incorporated into protein at a rate similar to the amino acid methionine (Sunde, 1997). Selenomethionine can be incorporated as Met into protein because Se and sulfur have similar atomic properties, including bond lengths and chemical reactivities, and the body does not distinguish between the two compounds (Sunde, 1997). According to Whanger (1996), when dietary levels of sulfur via Met are high, then the conversion of SM to selenocysteine is increased. Our diets were sufficient in Met, which indicates either a potential for the increased conversion of SM, the primary fraction of SY, to selenocysteine or the non-preferential absorption of SM into protein. The increase in conversion of SM to selenocysteine could lead to increased GPX-3 activity. Waschulewski and Sunde (1988) reported that SM was not used as effectively by rats for GPX activity when diets were deficient in Met, but muscle Se levels were increased 3.5-fold compared with rats fed diets adequate or high in Met.

The broilers previously fed SY maintained higher GPX-3 activity than those previously fed SS, and by d 16 (6 d after removal of the diets containing Se), broilers previously fed SS had higher GPX-3 activity than broilers previously fed the C-SBM diet. Plasma Se levels decreased in all broilers on d 13 but then seemed to plateau.

Broilers previously fed SY maintained higher plasma Se levels from d 13 to 19 compared with the broilers previously fed SS, and broilers fed SS had higher plasma Se levels relative to those previously fed the non-supplemented C-SBM diet. Our results for GPX-3 in which activity decreased over time as broilers were fed a Se-deficient diet are in agreement with Noguchi et al. (1973), who reported that GPX-3 activity is very responsive to an animal's dietary Se status. However, we are not aware of literature comparing GPX-3 and tissue Se concentrations over time in animals previously fed SS or SY. Our results during the depletion period seem to be in agreement with the kinetic flux model in the human by Patterson et al. (1989), Swanson et al. (1991), and Patterson and Zech (1992), as described by Sunde (1997). This model indicates that a considerable amount of organic Se fluxes into a very slow turnover plasma pool after cycling through both slow and fast turnover tissue pools. In contrast, only a minimum amount, if any, of the inorganic Se enters this same pool, which has a peak of greater than 50 hrs after absorption and a half-life of over 6 d. This would help explain how the broilers previously fed SY were able to sustain GPX-3 activity at higher levels than those fed SS.

Our results indicate that the breast tissue Se levels decreased over time after the broilers were removed from the diets containing Se. The broilers fed the SY had the highest breast Se concentrations on d 10, and their breast Se concentrations remained higher throughout the trial. This response is consistent with the accepted biochemistry of the organic Se sources as described above in that the organic Se tends to be deposited more in slow turnover tissues, such as muscle (Sunde, 1997). The broilers fed the SS had higher breast Se concentration compared with those fed

the C-SBM diet with no supplemental Se, but by d 22 (12 d after removal of the Se source from the diet), there was no difference in the breast Se levels of broilers fed SS or those fed the C-SBM diet with no supplemental Se. The increased breast Se from d 10 to 19 in broilers fed SS is not consistent with the kinetic model of the flux of Se because it indicates that practically no inorganic Se will enter slow turnover tissues. Our results suggest that either some of the SS is available for conversion to a Se analog of the TSAA, or that the indigenous SM from the natural feedstuffs was not used for other seleno-proteins and thus could be incorporated into muscle.

The broilers fed SY maintained greater liver Se levels through d 16 compared with those fed SS or the C-SBM diet, after which the liver Se levels were similar for broilers previously fed SY or SS. Liver Se levels slowly decreased from d 10 to d 13 for the broilers fed SS or SY before they plateaued on d 19. The liver Se levels of the broilers previously fed the C-SBM diet decreased dramatically after d 10 and plateaued by d 13 at lower levels than those fed either Se source. Our results seem to agree with the kinetic flux model (Sunde, 1997) in that 89% of absorbed organic Se is initially shuttled through the liver compared with 77% for the inorganic Se. Based on the recycling of Se, through the gastrointestinal tract and also through the slow and fast turnover plasma and tissue pools, it seems that more organic Se is recycled and returned to the liver than the inorganic Se.

The glutathione peroxidase enzymes, which include GPX-3, belong to a class of Se-specific proteins (Sunde, 1990). A unique characteristic of this class of enzymes is that selenocysteine is the Se moiety for these proteins, and so selenocysteine is essential for GPX-3 to function properly (Forstrom et al., 1978).

Because Se and sulfur have such similar chemistries, their metabolic pathways are thought to be similar. One distinct difference though is that sulfur compounds undergo oxidative pathways, whereas Se compounds follow reductive pathways (Sunde, 1997). In the metabolic pathway of Se, SM has at least three fates upon absorption: it can be readily incorporated into protein, or it can be synthesized into selenocysteine using serine via the transsulfuration pathway, or it can be converted into methylselenol via the transamination-decarboxylation pathway (Sunde, 1997). Unlike SM, SS seems to have only one fate and that is to be reduced to selenide via the glutathione and glutathione reductase pathways (Sunde, 1997). After reduction to selenide, SS has several options including being converted into usable seleno-proteins, such as selenocysteine (Sunde, 1997). Knowing this and combining it with the fact that the majority of the Se in SY is SM (Beilstein and Whanger, 1985), then we may be able to explain how SY can maintain higher levels of GPX-3 levels after removal from Se-supplemented diets. It would be logical that SS would be metabolized to selenide rapidly after absorption, because that is the only pathway available, thus it would potentially be used quicker for GPX-3 or at best stored in the liver or kidney for a short time. The SY, however, will behave more like SM and be split into any or all three pathways upon absorption. There is also likely to be some inorganic Se associated with the SY product, and if so, that fraction will enter the inorganic pathway and be used rapidly.

By d 10, the breast Se levels were three times higher in broilers fed SY than in broilers fed either of the other diets. There was no appreciable accumulation of Se in the breast of broilers fed SS or the diet with no supplemental Se, and as such

it does not seem that the breast tissue provided any Se to promote GPX-3 activity in these birds. From our results, it seems that the plasma and liver Se pools were used by the broilers fed SS or the diet with no supplemental Se to maintain GPX-3 activity, and as the liver and plasma were depleted of Se, GPX-3 activity subsequently decreased. There does seem to be a conservation of Se in the body once reserves reach a certain level as all tissues and plasma levels of Se and GPX-3 activity plateaued and remained constant throughout the remainder of the trial. Our results also indicate that the liver and plasma pools in the broilers fed SY are probably used first to maintain GPX-3 activity, but the Se that was stored in the breast also began decreasing, although slowly, by d 13. This decrease in breast Se level indicates that the protein was being turned over and the Se was being recycled and metabolized for other functions.

In conclusion, the results from these EXP indicate that Se from organic sources, such as SY, can be utilized for GPX-3 activity even after the Se has been stored in slow turnover tissues, such as muscle. Furthermore, these organic types of Se are more efficient in maintaining GPX-3 concentrations compared with inorganic sources, such as SS, when in a low-Se state.

## CHAPTER 6

### SUMMARY AND CONCLUSIONS

The purpose of this research was to compare the effects of inorganic and organic Se in diets for broilers and laying hens, and then to determine the fate of Se once it has been absorbed and deposited into various tissues. In the experiment using laying hens, egg percentage, percentage of shell-less eggs, feed intake, and feed efficiency were not affected by source or level of Se, but feed intake was increased in hens fed the control diet compared with those fed diets supplemented with Se. The percentages of dirty and cracked eggs were increased when hens were fed the organic Se. Albumin quality of eggs stored at 22.2°C was improved in hens fed the inorganic Se compared with those fed the organic Se, but there was no difference when the eggs were stored at 7.2°C. Egg Se levels were increased as Se supplementation increased, and eggs from hens fed either Se source were higher in Se content than those from hens fed the basal diet. The eggs from hens fed the organic Se were higher in Se than those from hens fed the inorganic Se.

In the second experiment, growth performance, carcass traits, and plasma glutathione peroxidase activity of broilers fed Se for 49 d were not affected by Se supplementation. However, muscle and plasma Se levels were higher in broilers fed organic Se compared with those fed the control diet or the diet with inorganic Se. The next experiments were conducted to validate a Se deficient diet. The results from these experiments indicated that a semi-purified diet containing 10% torula yeast and supplemented with Se provided similar growth performance in broilers as a corn-soybean meal diet. Furthermore, the broilers fed the 10% torula yeast diet

without Se supplementation had decreased plasma Se and glutathione peroxidase activity compared with those fed the control diet, but these levels returned to normal when Se was provided.

Our previous research had shown that organic Se was deposited into muscle tissue at a much greater rate than inorganic Se. Thus, the final experiment was conducted to determine if the broiler could use the Se in the tissue during times of Se deficit. Broilers were fed inorganic or organic Se for 10 d and then placed on a Se deficient diet. Growth performance was not affected by Se supplementation or by the low-Se diet. On d 10, plasma glutathione peroxidase activity and Se levels were similar for the broilers fed either Se source, but liver and breast Se levels were higher in broilers fed organic Se than in those fed inorganic Se. Broilers previously fed organic Se maintained higher plasma glutathione peroxidase activity, and plasma, liver, and breast Se levels compared with those previously fed inorganic Se or the control diet for a minimum of six days after being placed on a low-Se diet.

The objective of this research was to compare inorganic and organic sources of Se and to assess its effects once absorbed. The results of these experiments indicate that organic Se sources increased tissue and plasma Se levels without negatively affecting growth performance or plasma glutathione peroxidase activity compared with inorganic Se. These results also indicate that Se from organic sources are available for use in Se-dependent activities, such as glutathione peroxidase even after they had been stored in slow turnover tissues such as muscle.

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**APPENDIX**  
**LIST OF ABBREVIATIONS**

Item	Abbreviation
Average daily gain	ADG
Average daily feed intake	ADFI
Body weight	BW
Corn	C
Cornstarch	CS
Cytosolic glutathione peroxidase	GPX-1
Dextrose	D
Experiment	EXP
Gastrointestinal glutathione peroxidase	GPX-2
General abbreviation for glutathione peroxidase	GSH-Px
Light hours to dark hours	L:D
Monomeric glutathione peroxidase	GPX-4
Percentage of chill weight	PCW
Percentage of live weight	PLW
Plasma glutathione peroxidase	GPX-3
Selenium-enriched yeast	SY
Selenomethionine	SM
Sodium selenite	SS
Soybean meal	SBM
Thyroxine 5'-deiodinase-1, 2, and 3 enzymes	DI1, DI2, and DI3
Torula yeast	TY

## **VITA**

Robert L. Payne III was born in Tullahoma, Tennessee, on May 13, 1975. Robert was raised in Smyrna, Tennessee, by his parents, Bob and Wanda Payne. He graduated with honors from Smyrna High School in May, 1993. Robert began attending Middle Tennessee State University for his undergraduate education in August, 1993. He majored in animal science, with a minor in basic science. Robert graduated from Middle Tennessee State University in December, 1997. Robert moved to Baton Rouge and began working towards his master of science degree in animal science at Louisiana State University in August, 1998. Robert graduated from Louisiana State University in May, 2000, and then began working towards the doctor of philosophy degree in animal science also at Louisiana State University. That degree will be conferred at the August, 2004 Commencement.