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The Effect of Vitamin E, Selenomethionine and Sodium Selenite Supplementation in Laying Hens

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The Effect of Vitamin E, Selenomethionine and Sodium Selenite Supplementation in Laying Hens

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In trial 1, 3 levels of dl-α-tocopheryl acetate (0, 50, 100 IU/kg), and 3 levels of supplemental sodium selenite (SS) (0, 0.25, 0.50 ppm), were added to a corn-soybean meal basal diet to evaluate their effects on egg quality variables, and deposition in egg yolk. Adding 50 IU/kg dl- α-tocopheryl acetate in the diet lowered aged yolk pH. Alpha-tocopherol in yolks increased with increasing vitamin E. As Se level increased in the diet, yolk Se content increased. There was a vitamin E by Se interaction affecting yolk Se content, but the highest level of Se in the yolk achieved when using 0.5 ppm Se from either source with no vitamin E. Trial 2 was conducted to investigate the effects of using organic vs. inorganic Se on egg quality, egg yolk vitelline membrane strength, and glutathione peroxidase activity in the liver and shell gland of hens. Hens were fed a corn-soybean meal basal diet supplemented with 0, 0.2 ppm selenomethionine (SM), 0.2 ppm SS, 0.4 SM, or 0.4 ppm SS. Supplementing SS at 0.2 ppm or SM at 0.4 ppm had the same effect to improve the VMS. In trial 3, hens were fed the same dietary treatments as in the second trial and added to a semi-purified corn starch-soybean meal basal diet. Yolk Se content was higher in all treatments supplemented with Se from either source than the control diet. There was an interaction effect of Se source and level on albumen

Se content; albumen Se content increased when SM levels in the diet increased, whereas when SS levels increased in the diet, there was no increase in egg albumen Se content. In summary, our results indicate that vitamin E and Se supplementation from the organic and inorganic sources can be a good practice to increase some of the egg quality parameters, but more research need to be conducted when the basal levels of Se are low.

Key words: vitamin E, sodium selenite, selenomethionine.

Dedication

This dissertation is dedicated to my parents, who taught me that even the largest task can be accomplished if it is done one step at a time and if I use my time correctly. Thank you for instilling the importance of hard work and higher education, and thank you for your unconditional love, support, and guidance. I love you so much.

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CHAPTER 1

Literature Review

INTRODUCTION

Selenium (Se) is an essential micronutrient in the diet of many life forms including animals and humans. The biochemical role of Se was demonstrated in 1973 by Rotruck et al. when it was discovered as part of the enzyme glutathione peroxidase (GSH-Px). Glutathione peroxidase acts as an antioxidant to prevent cellular damage by free radicals produced as natural by-products of oxygen metabolism in the body. As a result, GSH-Px prevents development of chronic diseases such as cancer and heart diseases. In addition to its role in GSH-Px, Se has been shown to slow the aging process, prevent muscle disorders, aid in the metabolism of the sulfur-containing amino acids, allow for normal fetal development during pregnancy, ensure proper function of the thyroid gland, and stimulate immune function (Moustafa et al., 2003).

Traditionally, Se has been added to poultry diets via inorganic sources, such as sodium selenite (SS) (Na_2SeO_3). Research has shown that organic Se is more bioavailable than Se in SS (Cantor et al., 1982). Selenomethionine (SM) accounts for the largest portion of Se in Se yeast (Paton et al., 2002). Selenium yeast has been reported to be an excellent source of organic Se (Kelly and Powers, 1995). It is manufactured by growing a commercial Saccharomyces yeast strain in a sulfur deficient/Se rich medium that forces the yeast to incorporate Se in the form of SM. A study done by Payne et al. (2005) indicated that Se yeast or SM results in greater deposition of Se in eggs than does

SS; therefore, Se maybe more available for humans consuming these eggs. Results of current studies have provided evidence that organic forms of Se are generally safer and better absorbed (Edens, 2002). These developments raise questions as to which form of Se is best for dietary supplementation.

All the elements of the antioxidant system interact with each other, forming an efficient antioxidant defense. For example, dietary Se had a sparing effect on vitamin E, with the result that chickens given Se supplements had significantly increased plasma vitamin E concentrations (Surai, 2000).

The main role of vitamin E as an antioxidant is to prevent lipid oxidation which largely affects the deterioration of food products, and has adverse effects on color, flavor, nutritive value and even safety of food products (Burlakova et al., and 1998; Moak and Christensen, 2001). Early studies done on animals have shown that inadequate amounts of vitamin E result in anemia, reproductive failure, muscular dystrophy, and neurological disease (Leonhardt et al., 1997). Vitamin E supplementation is an effective way to alleviate the negative effects of stress on laying hens. Vitamin E along with selenium can act together to adverse the effects of heat stress (Sahin and Kucuk, 2001).

Data concerning effects of high levels of Se and vitamin E supplementation of the hens' diet on the deposition of these nutrients in the egg are few. In addition, the activity of GSH-Px in tissues as affected by the different sources and levels of selenium has not been studied thoroughly. Thus, our experiments may confirm that it is possible to produce eggs enriched with Se and vitamin E for human consumption through a targeted manipulation of hen diets, and can show the effect of vitamin E and Se from different

sources on egg production and quality parameters as well as the activity of GSH-Px in body tissues.

SELENIUM

History

In 1817 the Swedish chemist, Jons Jakob Berzelius, discovered a new element in red deposits while he was investigating illnesses in a sulfuric acid plant in Gripsholm. It was first given the name Selene, after the Greek moon goddess, before it was named selenium (Se) (Oldfield, 1999).

Around a century later, in 1957, Schwarz and Foltz demonstrated the effectiveness of the trace element (Se) in preventing liver necrosis in the rat (Schwarz and Flotz, 1957). Schwarz noticed that rats with liver necrosis could be protected by extracts of brewer's yeast (Saccharomyces cerevisiae), as well as by vitamin E supplementation. But vitamin E was effective only at high dosages, so this suggested to Schwarz that there was another more powerful protective factor that played a role, which he named 'Factor 3' and thought it was a vitamin, until a breakthrough in 1957 showed that it was selenium (Shwarz and Foltz, 1957). Since then, scientific information on Se chemistry, biochemistry and molecular mechanisms of action has been actively reported. Most attempts to uncover the biochemical functions of selenium were unsuccessful until 1973, when it was isolated as part of the enzyme glutathione peroxidase (GSH-Px) (Rotruck et al. 1973). This enzyme, present in the cytosol and mitochondrial matrix space, is involved in antioxidant defense mechanism at the cellular level. GSH-Px scavenges free radicals before they harm body tissues.

Chemistry

Selenium is classified in group VI in the periodic table of elements. It has both metallic and non-metallic properties and is considered a metalloid element, with atomic mass of 78.96 and an atomic number 34. Selenium exhibits allotropy, which means that it appears in many forms; including a red amorphous powder, a red crystalline material, and a grey crystalline form. In nature, Se exists in two chemical forms, the organic and the inorganic forms (Foster and Sumar 1997).

Elemental Se can be oxidized to +4 (SO_3^{-2}, selenite) or +6 (SO_4^{-2}, selenate) oxidation states, or reduced to -2 reduction state (selenide). So, inorganic Se can be found in different minerals in the form of selenite, selenate, and selenide as well as the metallic original form. Sodium selenite and sodium selenate are the most common inorganic forms (Carvalho et al., 2003). Selenium in the organic form includes selenomethionine, selenocysteine, amino acid chelates, yeast, and kelp bound Se.
In feedstuffs, organic Se exists in combination with the amino acids methionine and cysteine (Surai, 2002). The absorption of Se in plants depends on the soil levels of Se, which vary significantly depending upon geographical location (Reilly, 1996).

Distribution

Selenium distribution in the United States is variable. Eastern and Northwestern areas of the country have low Se in the soil and forages, especially areas bordering the Great Lakes; whereas Se amount in soils of the Midwest are considered adequate (Leeson and Summers, 2001a).

The concentration of Se in soils depends on many factors. In acidic soils, or poorly aerated soils, Se forms insoluble complexes with iron hydroxide and becomes poorly available. Total soil Se does not correlate to the amount of water soluble Se in soils, which is available for plants (Reilly, 1996), so the animal feed ingredients also vary in their Se content. As a result Se supplementation is important to maintain adequate amounts in the animal.

Sources

Selenium sources in food include Brewer's yeast and wheat germ. Animal foods considered adequate in Se include liver, butter, most fish, and lamb. Whole grains, nuts, and molasses are fairly good sources as well. Brazil nuts have high amounts of Se, and barley, oats, whole wheat, shellfish, shrimp, and oysters are rich in Se (The Carribian Food and Nutrition Institute, 2005).

The biological activity of Se in fishmeal is poor but represents one of the best natural sources of Se among the common poultry feedstuffs. The content of Se in other poultry feedstuffs varies from one place to another; for example, corn in Nebraska and South Dakota has 0.38 ppm Se. Soybean meal in the Midwest in general contains 0.1 ppm Se, but it contains has 0.54 ppm Se in Nebraska (Scott, 1973).

Recommendations

The RDA for Se in the U.S. is 55 µg/day for adult humans (Fisinin et al., 2009). Many people consume less than the RDA depending on their food source place of origin. In animals, Se intake also depends on their feed source. In the 1960s, the U.S. suffered

from Se-deficiency diseases that caused millions of dollars of losses in the livestock and poultry industry, which urged the need for new sources of Se and the use of supplements (Schrauzer and Surai, 2009).

In 1974, the FDA (U.S. Food and Drug Administration, 1974) approved additions of the inorganic sources of Se to feedstuffs, and since that time Se supplements have been allowed in different animal feeds. The amount of Se supplementation permitted in layer and breeder diets is 0.2 to 0.3 ppm (Leeson and Summers, 2001a). Poultry and livestock diets were approved for supplementation with Se yeast at 0.3 ppm (Federal Register, 2000, 2002).

Many studies have indicated a linear relationship between the Se in hen diets and the amount of Se in the egg. Davis and Fear (1996) indicated that hens supplemented with a total of 0.419 ppm Se gave a 7.1-fold higher Se content in eggs than hens supplemented with a total of 0.027 ppm Se. Se-enriched eggs can be a safe vehicle for human intake of Se that is consumed regularly in moderate amounts by the majority of the population as part of their traditional meals and can deliver 50% of the RDA for humans (Fisinin et al., 2009).

Metabolism and Excretion

Organic and inorganic Se are metabolized in different ways, but both must be converted to the common selenite and/or are further reduced to hydrogen selenide before Se can be inserted into body selenoproteins (Foster and Sumar, 1997) (Figure 1).

Selenomethionine (SM) is not synthesized in the body and must be provided from feed sources (Schrauzer, 2000). Once feed sources reach the intestine, SM is actively

transported through intestinal membranes during absorption and actively deposited in liver and muscle. There are two pathways for catabolism of SM. Selenomethionine can go through the transsulfuration pathway via selenocystathione to produce selenocysteine. It is then degraded by a decarboxylase into hydrogen selenide (Beilstein and Whanger, 1992). The other pathway involves transamination-decarboxylation (Mitchell and Benevenga, 1978).

The specific role of the chick duodenum in the digestion and absorption of the inorganic Se was shown by Apsite et al. (1993). Selenite is passively absorbed in the duodenum of the small intestine and the anterior ileum of the chicken (Pesti and Combs, 1976). Inorganic forms of Se, such as sodium selenite, are metabolized to hydrogen selenide via selenodiglutathione and glutathione selenopersulfide (Turner et al., 1998). Hydrogen selenide is the precursor for supplying Se in an active form that can be used for the synthesis of selenoproteins (Sunde et al., 1997). Further metabolism of hydrogen selenide involves methylation by S-adenosylmethionine to methylselenol, dimethyl-selenide and triethylselenomium ion (Foster et al., 1986).

Selenium binding proteins are found in the plasma; the most important one is glutathione peroxidase (GSH-Px). When Se intake exceeds the requirement, Se binds to these binding proteins and then methylated either to dimethyl selenide, or its further methylation step to trimethyl selenonium ion, which is the normal excretory product in the urine. If the intake exceeds that, then dimethyl selenide is excreted via air, which gives a garlic odor (Francesconi et al., 2004).

Deficiency

Selenium deficiency has been described in humans as well as many other species. A Se deficiency has been known to cause a disorder in humans known as Keshan Disease. Keshan disease was named after an epidemic outbreak in 1935 in Keshan County in China. This disease occurred in the Se-deficient soil areas (Cheng and Qian, 1990). It is characterized with cardiomyopathy that occurs with signs of congestive heart failure (Aro et al., 1994). Another disease found in humans as a result of Se deficiency is Kashin-Beck disease. Kashin-Beck is also prevalent in areas of the world with Se-deficient soils. A range of bone and joint deformations that develop during childhood and puberty characterizes the disease (Yang et al., 1993). Selenium deficiency can also affect muscle weakness and tenderness, and cardiomyopathy with decreased cardiac function (Marcus, 1993). Other possible health effects that have been reported in numerous studies indicate that Se-deficient patients are more prone to seizures, rheumatoid disease, arteriosclerosis, miscarriages, neurological disorders, depression, and even cancer (Ramaekers et al., 1994; and Rayman, 2000).

In poultry, Se-deficiency, especially when combined with a vitamin E deficiency, can be responsible for a range of avian diseases including exudative diathesis, nutritional encephalomalacia, and nutritional pancreatic atrophy (NPA) (Leeson and Summers, 2001a). Se-deficiency in chickens is also associated with impaired immunocompetence, reduced egg production, and increased embryonic mortality (Combs and Combs, 1986). Se-deficiency in poultry affects fertility and hatchability. Latshaw and Osman (1974) reported low fertility and hatchability when birds were fed a basal (low Se) diet, but this reproductive disorder could be corrected partly by vitamin E supplementation and

completely by Se-supplementation. Eggs from hens fed a very low level of Se were more likely to be infertile (12.6%), and more likely to have high embryonic mortality (29%) and lower hatchability (15%) (Latshaw et al., 1977).

Exudative diathesis (ED) in hatchlings from hens with no Se supplementation can also be elevated (Hassan et al., 1990). Exudative diathesis is a common problem in chickens deficient in Se and vitamin E. Exudative diathesis is attributed to increased capillary permeability due to endothelial cell failure in skeletal muscle (Combs and Scott, 1974). In ED muscles become pale, chicks stand with their legs far apart and a weeping dermatitis appearing as a green-blue lesion can be seen on their skin (www.worldpoultry.net). Supplementing the diet with Se rather than vitamin E is more effective in preventing the occurrence of ED. Vitamin E supplementation at 15 ppm was not enough to prevent ED while levels as low as 0.15 ppm of Se was sufficient (Hassan et al., 1990). Therefore, ED is considered to be a Se-deficiency syndrome in chickens (Leeson and Summers, 2001a).

Toxicity

Toxic levels of Se (10-20 ppm) are more than 100-fold higher the nutritional requirements. Usually Se doses lower than 3-5 ppm in animal feed are not associated with toxicity (Surai, 2002). The consumption of higher levels of Se can cause selenosis, characterized by hair loss, gastrointestinal upsets, white blotchy nails, fatigue, irritability, and mild nerve damage in both humans and animals (Koller and Exon, 1986).

In chickens, excessive amounts of Se can cause decreased growth rate, egg production, and hatchability. Ort and Latshaw (1978) found that egg production and

hatchability were decreased in breeder hens fed 7 ppm Se. In White Leghorn chickens fed a basal diet of 0.3 mg Se/kg supplemented with 0, 0.1, 0.5, 1.0, 3.0, 6.0 mg Se/kg as selenomethionine for 18 weeks no toxic effects were found even in the highest levels of Se (Moksnes, 1983). When Se as sodium selenate was supplemented in the feed from 0.1 to 9 ppm, hatchability of fertile eggs was significantly decreased in treatments supplemented with 5 ppm Se or higher, egg weight with 7 ppm or higher, and egg production was only decreased with supplementation at 9 ppm of Se (Ort and Latshaw, 1978).

Glutathione Peroxidase

Glutathione peroxidase (GSH-Px) is a tetrameric protein with four identical subunits, each with a molecular weight of ~ 23,000 Da and each containing one Se atom (Sunde, 1993). Glutathione peroxidase is found in all body tissues where oxidative processes occur (Kohrle et al., 2000). It reduces hydrogen peroxides (H_2O_2) and other peroxides to water and alcohols, which prevents production of reactive oxygen species. Maintenance of the cellular redox state is another important function of the GSH-Px; it has a role in differentiation, signal transduction and regulation of proinflammatory cytokine production (Ursini, 2000). Glutathione peroxidase participates in regulating biosynthesis of leukotrienes, thromboxanes, and prostaglandins, which all work in the modulation of inflammatory reactions (Kohrle et al., 2000). It has been shown that the cell's first line of antioxidant defense is based on activity of three enzymes: superoxide dismutase (SOD), GSH-Px and catalase. The importance of GSH- Px in antioxidant

protection of tissues has been increasingly studied. The major type of GSH-Px is Se dependent, so Se in animal nutrition has attracted considerable attention (Mahan, 1999).

There are different forms of glutathione peroxidases in the body depending on their location: 1) Phospholipid GSH-Px, 2) Plasma GSH-Px, 3) Gastrointestinal GSH-Px, 4) Cytosolic GSH-Px, and 5) Specific sperm nuclei GSH-Px. Generally, these different forms of GSH-Px act together in concert to provide antioxidant protection at the different sites of the body (Kohrle et al., 2000).

It was not until 1973 that Rotruck et al. discovered that selenium was an integral part of the enzyme glutathione peroxidase (Rotruck et., al 1973). The activity of GSH-Px depends on Se supplementation in the diet. So the activity of glutathione peroxidase in certain tissues can be used as an index of selenium adequacy (Paynter, 1979).

In a study done by Surai (2000), GSH-Px activity in the liver of day old chicks was dependent on Se level in the maternal diet. Low Se content was associated with decreased Se in the egg yolk, and consequently liver Se-GSH-Px activity in newly hatched chicks was significantly decreased. Similar results were found by Bunk and Combs (1981) when they found that chicks produced from hens fed a basal diet with low Se and low vitamin E diet had low activities of GSH-Px in their plasma and their pancreas at hatching. On the other hand, when Se was supplemented, Se-GSH-Px activity increased in the liver and pancreas of chicks (Surai, 2002).

VITAMIN E

History

Vitamin E was discovered in 1922 by American physician Herbert Evans and his assistant Katherine Bishop at the University of California (Evans, 1963). They discovered it when they noticed that rats reared on a basal diet stopped reproducing until they were given a substance isolated from vegetable oils, and with that substance they could produce healthy and strong offspring (Leeson and Summers, 2001b). Vitamin E is a group name that includes a number of active compounds. There are eight naturally occurring forms of vitamin E, and these are divided into two groups according to whether the side chain of the molecule is saturated or unsaturated. The saturated forms are referred to as tocopherols (McDowell, 1989), and they are designated as α, β, γ, and δ. The unsaturated tocorienols were discovered by Green et al. and Pennock et al. in 1960-1964 (Wang and Quinn, 1999). Tocorienols also have four forms α, β, γ, and δ. Of all these forms, α-tocopherol is the most biologically active and most widely distributed.

Many studies have been made on a variety of experimental animals to elucidate vitamin E's importance in the functioning of most body tissues and to prevent many deficiency diseases. Vitamin E is required for normal fertility in the rooster and normal reproductive performance of the hen; a deficiency with chicks can lead to lipid degeneration and hemolysis. With an acute deficiency, chicks may start developing encephalomalacia, exudative diathesis, or muscular dystrophy. Some specific dietary changes can alleviate one or more of those deficiency diseases. Synthetic antioxidants can prevent encephalomalacia, Se can prevent exudative diathesis, and cystine can

prevent muscular dystrophy, all of these still referred to as vitamin E deficiency diseases and can be prevented by vitamin E supplementation (Leeson and Summers, 2001b).

Chemistry

The exact structure of α-tocopherol was elucidated by Fernholz and the Swiss chemist Karrer and the name 'vitamin E' was suggested by Sure and was adopted by Evans and Bishop (Surai, 2002). The word tocopherol is derived from the Greek words *tokos* meaning birth, and *pherein* meaning to bear or carry (Surai, 2002).

The chemical structure of vitamin E was discovered by Fernholz (Fernholz and Finkelstein, 1938). The term vitamin E is the general description used for all tocol and tocotrienol derivatives which include α, β, γ, and δ-tocopherol and α, β, γ, and δ-tocotrienol. The molecular weight of α-tocopherol is 430.69 and its UV absorption is 292-294 nm with absorption of 1% solution in ethanol in a 1 cm cuvette of 72-76 (Machlin, 1991).

The d form of α-tocopherol is slightly viscous, pale yellow oil, insoluble in water but soluble in oils, fats and organic solvents. It has a melting point of 2.5-3.5°C and a boiling point of 200-220°C. It can be purified by molecular distillation and has a maximum absorption of 295 μm and minimum absorption of 267 μm. Vitamin E is synthesized from trimethylhydroquinone and isophytol, resulting in approximately equal portions of the 8 possible isomers (Leeson and Summers, 2001b).

Vitamin E is very unstable, prone to oxidative destruction by minerals and by unsaturated fatty acids in a diet. Esterification makes it more stable. The commercial supplements are usually d-α-tocopherol acetate, or dl-α-tocopherol acetate. These

acetates are prepared by reacting the tocopherol with acetic anhydride. The α-tocopherol acetate is an even more stable form when encased in gelatin beadlets (Leeson and Summers, 2001b). One IU of vitamin E has the activity of 1 mg of synthetic dl- α-tocopheryl acetate, 0.735 mg d- α-tocopheryl acetate, 0.671 ng d- α-tocopherol, or 0.909 mg dl- α-tocopherol (NRC, 1994). (Figure 2).

Sources and Recommendations

Tocopherols are present in oil seeds, leaves, and the green parts of plants; mainly in the chloroplasts of plant cells. They are concentrated in the leaves of plants rather than the roots, and in the dark mature leaves rather than the pale immature leaves (Diplock, 1985). Tocotrienols are found in the bran and germ fractions of seeds and cereals (White and Xing, 1997). Vitamin E level in feed depends on the crop location, fertilization, plant health and weather. The requirement for vitamin E in poultry is highly variable and depends on the concentration and type of fat in the diet, the concentration of Se, the presence of prooxidants and antioxidants. But NRC recommends 5 IU/kg of feed for a Leghorn-type laying hen consuming 100 g/d (NRC, 1994).

Metabolism and Storage

Vitamin E absorption follows the same pattern as fat absorption, which includes emulsification, solubilisation, diffusion across the unstirred water layer, permeation through the membrane of the enterocytes, incorporation into lipoprotein particles and release into the circulation via the lymphatic system in mammals or the portal system as in the avian (Cohn, 1997). Both bile and pancreatic lipase are essential for maximum

absorption. When the acetate form is used, pancreatic esterase helps in the initial cleavage. The α-tocopherol is absorbed with fatty acids as a lipid-bile-lipase micelle. Absorbed tocopherol is transported to the liver, as fats, via the portal vein in the avian. Absorbed tocopherol then binds lipoproteins that transfer them to the liver and most fat depots where they are stored (Brigelius-Flohe and Traber, 1999). Most of the ingested β, γ, and δ-tocopherol is secreted into bile or is not taken up and excreted in the feces (Drevon, 1991).

Deficiency

Young animals on vitamin E deficient diets are more susceptible to vitamin E deficiency compared to adult animals (Packer and Landvik, 1989). The three main vitamin E deficiency symptoms in chicks are encephamalacia, exudative diathesis, and muscular dystrophy. In mature birds fed low levels of vitamin E for prolonged periods, no clinical symptoms have been noted; however, decreases in egg production and hatchability are observed. Testicular degeneration is also noted in mature males fed a deficient diet for 6-8 weeks (Leeson and Summers, 2001b). Based on the concept of an integrated antioxidant system, vitamin E recycling and interaction with other antioxidants including Se, ascorbic acid, and glutathione can prevent vitamin E deficiency symptoms (Surai, 2002).

Toxicity

Vitamin E is one of the least toxic of all vitamins where signs are non-specific and most likely related to impairment of absorption of vitamins A and D, or fat itself.

(Leeson and Summers, 2001b). Many reviews have been published about vitamin E in excess in the diet and they all agreed that vitamin E is not toxic for humans and animals. Safe doses for animals are more than 100 times greater than the physiological requirements (Kappus and Diplock, 1992; Hathcock, 1997, and Diplock et al., 1998).

THE ROLE OF ORGANIC AND INORGANIC SELENIUM, AND VITAMIN E IN LAYING HENS

Vitelline Membrane Strength

The vitelline membrane of the egg separates the yolk from albumen. It is also the last barrier to microorganisms invading the yolk. Structural integrity of the vitelline membrane is important to prevent microorganisms from entering nutrient-rich yolk (Tan et al., 1992). Recently, vitelline membrane strength (VMS) has received attention in the egg-breaking industry. In the egg-breaking industry, liquid egg products consist of liquid whole egg, egg yolk, or egg albumen. Egg albumen is a foaming agent used in the baking industry and is dependent on the quality of albumen proteins. A slight contamination of egg albumen with egg yolk causes reduced foaming ability and loss for the egg-breaking companies. So the strength of the vitelline membrane is important to produce high quality egg albumen for egg producers. Yolk vitelline membrane has an essential role in embryogenesis as well; the sperm has to penetrate the membrane for fertilization to occur (Sim et al., 2000).

The structure of the vitelline membrane is composed of two distinct layers with different compositions; the inner layer, lamina perivitelline, which is 1.0 to 3.5

micrometers thick, is in contact with the yolk, and the outer layer, lamina extravitelline, which is 3.0 to 8.5 micrometers thick, is in contact with the albumen (Jensen, 1969).

Many factors can affect the quality and strength of the vitelline membrane, with storage duration and temperature being the most important. Albumen pH plays a role in the quality of the membrane as well (Feeney et al., 1956; and Kato et al., 1979).

To measure VMS, many methods have been developed. Kirunda and McKee (2000) determined VMS using a texture analyzer (TA). The idea of this method, as well as the other developed methods, involves creating rupture in the vitelline membrane by application of pressure on the yolk (Fromm and Lipstein, 1964).

In a study done by Monsalve et al. (2004), high dietary amounts of vitamin E (150 IU/kg) significantly ($P = 0.001$) improved VMS of fresh eggs. There was no significant difference in VMS of aged eggs by treatment, and the addition of selenium had no significant effect on VMS as well. Another study done by Kirunda et al. (2001) on the effect of vitamin E on egg quality during heat stress found that VMS declined in birds receiving the lowest vitamin E level of 20 IU/kg compared to 60 and 120 IU/kg. Froning et al. (1982) also found that 451 IU vitamin E/kg feed resulted in the highest VMS throughout the laying cycle compared with a diet supplemented with 231 IU/kg only. Researchers theorize that vitamin E as a fat-soluble vitamin is accumulated in the vitelline membrane and is responsible for its strengthening by functioning as an antioxidant (Halliwell and Gutteridge, 1989).

Egg Production and Quality Parameters

In a study done by Canan et al. (2007), egg production in laying hens in a heat stressed group and a non-heat stressed group both increased significantly ($P < 0.05$) with the supplementation of dietary vitamin E. These results agreed with the findings of Puthpongsiriporn et al. (2001) and Bollenger-Lee et al. (1999) who also showed that supplementation of vitamin E significantly increased egg production in laying hens exposed to heat stress.

A change in egg quality can be affected by many factors, including stress, age, and diet of the bird. In a trial done by Pappas et al. (2005), broiler breeders were fed 4 diets: soybean oil with no added Se, soybean oil with Se, fish oil, or fish oil with Se. Selenium used was from an organic source. Eggs from prepeak and peak production were stored for 2 weeks under typical conditions, and then Haugh units (HU) and pH, shell characteristics, egg components, weight, Se content, and fatty acid profile were measured. Albumen HU decreased with storage, although high Se treatments had greater HU compared with the low Se treatments. The reduction of HU as a result of storage is not new information (Scott and Silversides, 2000). The mechanism behind the decrease in HU is that carbon dioxide is lost from the egg contents by diffusion and pH of the albumen rises as a result (Williams, 1992; Brake et al., 1997). In their study, albumen pH rose from 8.95 to 9.5 after 14 d storage. The increased pH results from the dissociation of 2 proteins in the albumen (lysozyme and ovomucin) which in turn reduces the viscosity of the albumen and the HU.

Organic selenium can also affect egg shell quality. Paton and Cantor (2000) showed an increase in the shell breaking strength as a result of feeding organic Se to

Babcock laying hens at 80 weeks of age. Molecular mechanisms of the Se effects on shell formation are not well understood.

Deposition in the Egg

There are published data demonstrating a linear relationship between dietary dl-α-tocopheryl acetate level and egg yolk concentration of α-tocopherol. Jiang et al. (1994) found 390 mg/kg α-tocopherol in the egg yolk when feed was supplemented with 400 IU dl-α-tocopheryl acetate. Frigg et al. (1992) reported a concentration of 700 mg/kg α-tocopherol in the egg yolk when including 320 IU dl-α-tocopheryl acetate in the feed.

Utterback et al. (2005) studied the effect of supplementing organic Se in diets of laying hens on egg selenium content and found that the use of organic Se in laying hen diets was very effective for increasing the Se content of eggs. Eggs from the hens fed the Se-enriched diets had higher ($P< 0.01$) Se concentrations than did eggs from hens fed the basal diet at both 4 and 8 wk of the experiment. The organic Se diet also yielded egg Se levels that were significantly higher ($P< 0.01$) than those from the sodium selenite diet at both 4 wk and 8 wk.

The effect of using different sources of dietary Se on egg Se content has been studied by many researchers. Selenium, as with many other minerals, is preferentially deposited in the yolk compared with albumen. This is a consequence of mineral-binding lipoproteins in the formation of egg yolk (Richards, 1997). The efficiency with which Se is incorporated into the egg yolk is dependent on the concentration of dietary Se, with the Se at lower dietary inclusion levels being taken up most efficiently and preferentially deposited in the yolk. When the hens are fed a high Se diet, Se is incorporated less

efficiently into the egg and is partitioned equally between the yolk and the albumen (Pappas et al., 2005). The concentration of Se in the egg decreased as the age of the bird increased in the same study (Pappas et al., 2005). They suggested that this decrease of egg Se was not explained by the increased egg mass with older birds but by the less efficient mechanism by which Se was absorbed and deposited with age. As more Se is required for the operation of the antioxidant system and immune response, the demand on Se elsewhere maybe higher in the older hen than at younger ages.

Increasing the Se content of eggs can be beneficial for human consumption and for hatching eggs to raise the Se status of embryos and chicks (Cantor and Scott, 1974). Studies on the sparing effects of vitamin E and Se on each other have also been reported. Vitamin E accumulation in egg yolk reflected its level in the breeder diet and varied with Se supplementation (Surai, 2000). Dietary organic Se significantly increased the vitamin E level in the yolk, but no further increase in vitamin E accumulation in the egg yolk was noticed when a combination of Se and increased vitamin E were supplemented.

OBJECTIVE OF THE STUDY

Although research have shown the importance of vitamin E and Se in the laying hen industry at higher levels, NRC (1994) still has the requirement of vitamin E set as 5 IU/kg and Se as 0.06 g/kg of feed for a Leghorn-type laying hen consuming 100 g/d. Eggs enriched with vitamin E and Se can be a good and easy vector for the delivery of these essential nutrients for human health as well, so more research should be done in this area to convince costumers to go for designer eggs.

This dissertation combines three experiments conducted using different levels of vitamin E and organic and inorganic Se for the following objectives:

1) To test the effect of adding higher levels of Se and vitamin E than NRC, as well as different sources of Se on the production parameters of laying hens and egg quality.

2) To test the activity of GSH-Px in the liver, magnum and shell gland of laying hens, and to measure the strength of the vitelline membrane, as affected by supplementation of different levels and sources of Se in the hens' diet. More emphasis should be given to test the effect of Se on vitelline membrane strength, since the egg-breaking industry is growing and not much research has been conducted on this area.

3) To test the effect of Se and vitamin E supplementation on their deposition in the egg to produce eggs enriched with both nutrients for human consumption.

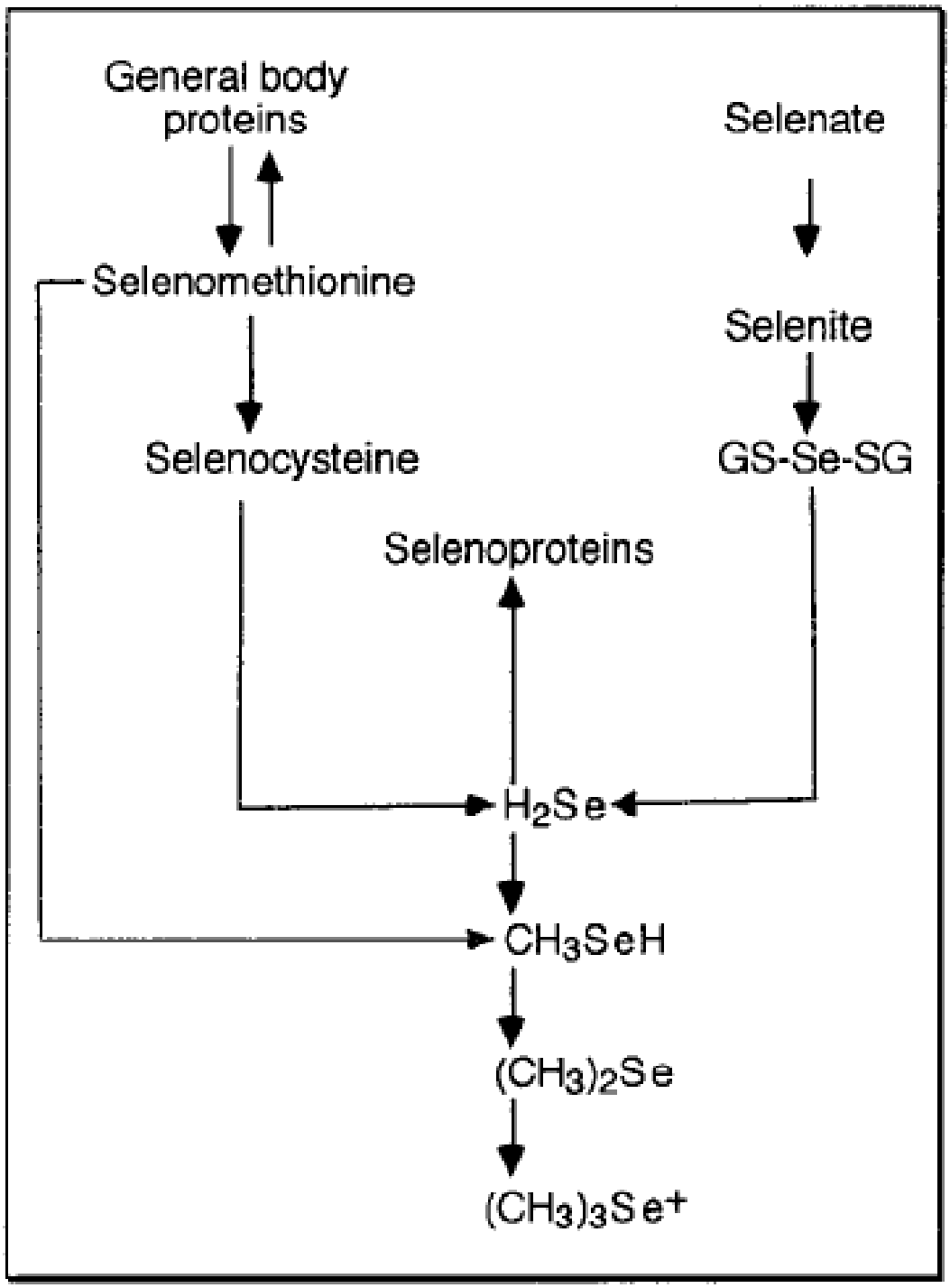

Figure 1.1 Metabolism of selenomethionine, selenite, and selenate (Shrauzer, 1998).

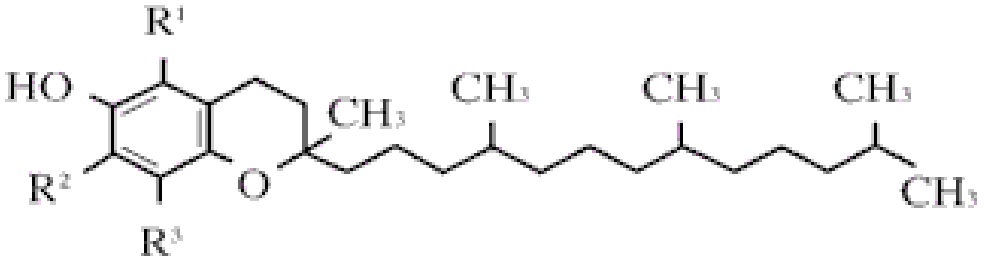

Figure 1.2 α-tocopherol structure (notice the saturated long hydrocarbon chain attached to the chromanol ring structure in the left) (Leeson and Summers, 2001).

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CHAPTER 2

Improving Egg Quality and Nutrient Value with Vitamin E and Selenium Supplementation in Laying Hens above NRC Requirements

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ABSTRACT The objective of this study was to assess the effects of dietary vitamin E and selenium (Se) supplementation at levels higher than the NRC requirements for laying hens on egg production and quality parameters and their deposition in egg yolk. A total of 216 White Bovan hens, 25 wks old, were fed the experimental diets for 26 weeks. Three levels of dl-α-tocopheryl acetate (0.0, 50, 100 IU/kg diet), and three levels of supplemental sodium selenite (SS) (0.0, 0.25, 0.50 ppm), were combined in a factorial design and added to a corn-soybean meal basal diet. Four eggs from each treatment were taken three times during the experiment to determine yolk α-tocopherol and Se contents. Dietary treatments had no effect on feed intake (P = 0.3656), egg production (P = 0.9761), hen weight gain (P = 0.8155), egg weight (P = 0.7104), Haugh units (P = 0.7076), specific gravity (P = 0.9908), and fresh (P = 0.1903) and aged albumen pH (P =

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0.1709). Supplementation with 0.25 or 0.5 ppm SS increased fresh yolk pH compared to treatments with no SS supplementation (6.059 and 6.061 vs. 6.027, respectively) (P = 0.0121). Aged yolk pH was affected by vitamin E level in the diet; adding 50 IU/kg dl-α-tocopheryl acetate in the diet significantly lowered pH to 6.180 (P = 0.0083). Alpha-tocopherol in yolks increased significantly (44.07, 192.13, 273.62 µg/g) with increasing vitamin E content in the diet from 0, to 50, and 100 IU/kg (P < 0.0001). There was a vitamin E by SS levels interaction affecting yolk Se content, with the highest level of Se in the yolk of 1213.75 ppb achieved when using 0.5 ppm SS with no vitamin E (P = 0.0058). In summary, using higher levels of vitamin E and SS than the NRC requirements had no significant effect on egg production parameters, but with increasing vitamin E level in the diet, aged yolk pH decreased, and α-tocopherol content of yolk increased significantly, and supplementation with the highest level of Se with any vitamin E combination always gave higher yolk Se content.

Key words: sodium selenite, egg production, vitamin E.

INTRODUCTION

The generation of free radicals and lipid peroxidation can contribute to the development of different diseases in humans as well as animals, with a decrease in the live performance and product quality in poultry. Selenium (Se) along with vitamin E work as antioxidants to prevent cellular damage by free radicals produced as natural by-products of oxygen metabolism in the bird (Surai, 2000).

Selenium essentiality was demonstrated in 1973 when it was discovered as part of the enzyme glutathione peroxidase (GSH-Px) (Rotruck et al., 1973). Among the main biological roles of Se in laying hens are (1) as an antioxidant to prevent oxidative stress, (2) to support proper thyroid function and (3) development and maintenance of immunocompetence (Surai, 2002). In addition to its role in GSH-Px, Se has been shown to slow the aging process, prevent muscle disorders, aid in the metabolism of the sulfur-containing amino acids, ensure proper function of the thyroid gland, and stimulate immune function (Moustafa et al., 2003).

In addition to the well-known function of vitamin E in chicken reproduction, vitamin E works as a major fat-soluble antioxidant, which breaks the chain reaction of lipid peroxidation. Early animal studies showed that inadequate amounts of vitamin E result in anemia, reproductive failure, muscular dystrophy, and neurological diseases (Leonhardt et al., 1997). Vitamin E requirements in laying hens and other food producing animals have been established to avoid clinical symptoms of deficiency (NRC, 1994). It has been proposed that the recommended levels of dietary vitamin E should vary depending on the susceptibility to oxidation rather than the amount needed to prevent signs of deficiency and mortality (Wang et al, 1996).

Balanced diets fed to highly productive lines of birds are the basis of efficient poultry production. In this respect, Se and vitamin E as antioxidants interact with each other to form an efficient antioxidant defense mechanism and when both are supplemented to birds they play an important role in maintaining bird health, productivity, and reproductive characteristics (Surai, 2000).

In order to combat the increased levels of free radicals resulting from stressful conditions in today's intensive poultry production systems, vitamin E and Se supplementation in the laying hen diets should increase more than the 1994 NRC recommendations. Thus, the objective of this study was to evaluate the effect of supplementing different levels of vitamin E and inorganic Se (sodium selenite- SS) well above the basal NRC recommendation on egg production and quality parameters and to produce eggs enriched with vitamin E and Se to increase human consumption of Se in an indirect way, as Se plays an important role in human health and prevention of disease.

MATERIALS AND METHODS

Birds and Housing

A total of two-hundred-sixteen Single Comb White Leghorn hens (Bovans White)[3], 18 weeks old, were obtained from a commercial laying hen operation and were transported to the Animal Science Department, F- House, at the University of Nebraska-Lincoln, October 2006. Animal care for this experiment complied with procedures approved by the University of Nebraska Institutional Animal Care and Use Committee (IACUC) (Protocol # 06-10-044D). Hens were fed a corn-soybean meal basal diet for 7 weeks before the start of the trial.

Two phases of feed were fed to the hens during the study, Phase I of the diet from 25-46 weeks of age and Phase II from 47-50 weeks of age for a total of 26 weeks. Hens were maintained on a 16: 8 hr light:dark cycle during the trial. Each bird had approximately 500 sq cm of floor space in stacked deck manure belt cages (Farmer

[3] Kumm's Kustom Pullets, NE, U.S.A.

Automatic with dimensions of 50 X 40 cm)[4]. Water was supplied *ad libitum* by nipple drinkers and the cage unit was located in a windowless, ventilated room.

Experimental Design

Hens were randomly assigned to 54 cages in a single laying hen unit with four hens per cage. Cages were blocked by side, north and south, each side with a total of 27 cages, and tier, each tier with a total of 9 cages, in a 3x3 factorial arrangement of 3 levels of supplemental dietary dl-α-tocopheryl acetate[5] (Calculated: 0.0, 50, or 100 IU/kg), and 3 levels of supplemental inorganic sodium selenite (SS)[4] (Calculated: 0.0, 0.25 or 0.50 ppm). Each dietary treatment was assigned to 6 replicate cages. Nine premixes were formulated to meet the hens' basic vitamin and mineral needs with the experimental levels of vitamin E and SS and then added to a corn-soybean meal basal diet.

Diets

Table 1 shows the diet composition and nutrient content of the basal corn-soybean meal diets for Phases I and II. Diets were formulated to be isocaloric to provide 2775 and 2820 kcal ME/kg during Phases I and II, respectively, and isonitrogenous to provide 16.7 and 16.5 % crude protein (CP) during Phases I and II, respectively. Hens were given *ad libitum* access to feed (100-110 g of feed per hen per day) and water during the study.

The 9 vitamin and mineral premixes were formulated and added to the diets during Phases I and II of egg production according to Bovans breeder's manual

[4] Farmer Automatic: P. O. Box 39 Register, Georgia, 30452, U.S.A.
[5] International Nutrition: P. O. Box 27540, 7706 I Plaza, Omaha, NE, 68127, U.S.A.

recommendations (Centurion Poultry, Inc.)[6] to meet the National Research Council (NRC) (1994) nutrient requirements for laying hens. Vitamin E and Se were weighed, along with the other minerals and vitamins and premixes were mixed in a vertical style mixer for 10-15 minutes (Table 2). Each premix was then added to 150 pounds of basal diet for each dietary treatment and mixed again for 10-15 minutes. Dietary samples were collected for each phase of diet formulation and were subsequently ground using a 1-mm screen Tecator cyclotec grinder[7] and stored in a -20°C freezer until chemical analysis was performed. All diets were analyzed for protein, Ca, phosphorus, vitamin E, and Se. Dietary α-tocopherol was analyzed in Midwest labs[8], and dietary Se was analyzed in Alltech labs[9].

Measurements

Hen and Egg Parameters

Data collected included percent daily hen egg production and daily feed intake. Both egg production and feed intake were calculated on a hen/day basis. All available eggs from 1 day's egg production were used to measure egg weight weekly, and two eggs with similar weight were picked for Haugh units (Haugh, 1937) measurements once every two weeks; an index of albumen quality, that is calculated from egg weight and albumen height, which is measured in the middle of the thick albumen, equi-distant from

[6] Centurion Poultry Inc.: P. O. Box 591/ 1095 Washington Road, Lexington, GA, 30648, U.S.A.
[7] Tecator Cyclotec Grinder: 1093 Sample Mill, Tecator, Hoganas, Sweden.
[8] Midwest Labs: 13611 B St., Omaha, NE, 68144, U.S.A.
[9] Alltech Labs: 3031 Catnip Hill Pike, Nicholasville, KY, 40356.

the outer edge of the albumen and yolk. Egg weight and Haugh units were recorded using Technical Services and Supplies (TSS) eggware[10].

$$HU = 100 \log (h-0.01*5.6745(30w^{0.37}-100) +1.9)$$

where

- HU = Haugh units.
- h = observed height of the albumen in millimeters.
- w = weight of egg in grams.

Specific gravity was analyzed every other week by submerging all collected eggs in buckets with graded NaCl (sodium chloride) salt solutions (1.070, 1.075, 1.080, 1.085, 1.090, 1.095 and 1.100) to determine the concentration at which the eggs would float. Hen weight was recorded monthly by averaging individual hen weights from each cage. Hen mortality was recorded daily during both phases of the experiment. A total of 8 hens died during the experiment. Production parameters such as feed intake and egg production were adjusted for hen mortality.

Egg yolk and albumen pH

Yolk and albumen pH were measured once every two weeks on fresh eggs and on eggs aged for two weeks in a cooler at 7°C. Six eggs per treatment were collected for pH measurements every two weeks. The yolk was separated from albumen using an egg separator. Yolk and albumen were poured into different glass beakers and homogenized with a stirring bar; then the sensing bulb of a glass pH electrode connected to a pH meter was used to measure pH.

[10] Technical Services and Supplies (TSS): York, England.

Egg Vitamin E and Selenium Analysis

Four eggs per treatment were collected for vitamin E and Se analysis 5 times during the trial (month 1, 2, 3, 4, and 5 of the trial). Yolks from months 1, 3, and 5 were utilized for vitamin E analysis. The yolks were separated from the albumen using an egg separator, homogenized, freeze dried, and ground by hand for vitamin E analysis. Alpha-tocopherol in yolks was analyzed in the labs of the Food Science Department at the University of Nebraska- Lincoln according to the methods of Carpenter (1979). Normal phase HPLC[11] chromatography was used for the analysis, in which the stationary phase used was the silica gel column and the mobile phase was isopropanol added to hexane at a ratio of 98:2 hexane:isopropanyl alcohol. Vitamin E analysis in the diet was analyzed at Midwest labs[8] using liquid chromatographic methods (A.O.A.C, 2007). Freeze dried yolks from months 2 and 4, and diet samples were analyzed for Se in Alltech labs[9] using a PSA Millenium Excalibur system, which uses continuous flow vapour/hydride generation atomic fluorescence to analyze for Se (Wallschlager et al., 2001).

Chemical Analysis

Dietary samples were collected for each phase of feeding and were subsequently ground using a 1-mm screen Tecator cyclotec grinder[7]. All diets were analyzed[12] for Ca (927.02), P (965.17), and crude protein (988.05) by A.O.A.C procedures (A.O.A.C, 1984). Dietary N was determined using the Kjeldahl method as established by the Association of Official Analytical Chemists (A.O.A.C., 1984). The N content in the diet was multiplied by 6.25 to determine protein content of the diet.

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[11] Lab Extreme, INC Kent City, MI, 49330, U.S.A.
[12] All diets analyzed according to the Association of Official Analatical Chemists (1984).

Statistical analysis

All data were analyzed as repeated measures using the GLIMMIX procedure of SAS (SAS 9.2, 2008). The experimental design was a repeated measures, randomized complete block design. Blocking was implemented in order to reduce the effect of temperature variation in the cage unit. Blocks were considered a random effect, dietary treatments were considered fixed. A 3 x 3 factorial arrangement of treatments was implemented with three levels of supplemental dl-α-tocopheryl acetate (0.0, 50, 100 IU/kg), and three levels of supplemental sodium selenite (0.0, 0.25, 0.5 ppm). Cages were considered the experimental units, with 4 hens per cage. Average values for the variables were generated and subsequently analyzed separately to determine differences between combinations of treatments. Repeated measures were done to measure treatment effect over time and the possible treatment by time interactions. Repeated measures were also done to identify possible covariance patterns in the repeated measurements and to determine the appropriate model to describe the time and measurement relationship. The appropriate covariance pattern for model fit was selected for each measurement using the information criteria (AKAIKE). Data were tested for the main effects of vitamin E, selenium, time, and their interaction. The following model was used to determine differences between treatments groups

$$Y_{ijklm} = \mu + R_{k+} \alpha_i + \beta_j + \alpha\beta_{ij} + \tau_l + \alpha\tau_{il} + \beta\tau_{jl} + \alpha\beta\tau_{ijl+} \varepsilon_{ijklm}$$

Where

Y_{ijklm} = Variable measured.

μ = Overall mean.

R_k = Effect of k^{th} block.

α_i = Vitamin E level effect.

β_j = Selenium level effect.

$\alpha\beta_{i\,j}$ = Interaction effect of vitamin E and Se levels.

τ_l = Time effect.

$\alpha\tau_{il}$ = Interaction effect of vitamin E level and time.

$\beta\tau_{jl}$ = Interaction effect of selenium level and time.

$\alpha\beta\tau_{ijl}$ = Interaction effect of vitamin E and selenium levels and time.

ε_{ijklm} = Residual error.

The separation of means was done using LS means statement with pdiff option.

The data for vitamin E and selenium content of yolks were analyzed using the model

$$Y_{ijkl} = \mu + R_{k+} \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijkl}$$

Where

Y_{ijkl} = Variable measured.

μ = Overall mean.

R_k = Effect of k^{th} block.

α_i = Vitamin E level effect.

β_j = Selenium level effect.

$\alpha\beta_{i\,j}$ = Interaction effect of vitamin E and Se levels.

ε_{ijkl} = Residual error.

The separation of means was done using LS means statement with pdiff option.

RESULTS

Table 1 shows the diet composition and nutrient content of the standard corn-soybean meal basal diet for Phases I and II of the production cycle and the analyzed protein, Ca, and P. Phase I of the diet was different from Phase II in the nutrient composition of energy and protein values. The calculated energy value in Phase I was 2775 kcal/kg, whereas in Phase II, it was 2820 kcal/kg. The calculated protein value in Phase I was 16.70%, whereas in Phase II it was 16.50%. Methionine value was 0.47% in Phase I and it decreased 0.10% in Phase II. Basal α-tocopherol and Se levels were the same during both phases; basal calculated vitamin E in the diet in both phases was 20 IU/kg, and basal calculated Se in the diet was 0.20 ppm as shown in the table.

Table 2 shows the dietary vitamin E and Se combinations and the analyzed vitamin E and Se results that were done by Midwest labs[8] and Alltech[9], respectively. Analyzed vitamin E content was zero for the non-supplemented treatments (Treatments 1, 2, 3). When vitamin E was supplemented at 50 IU/kg (Treatments 4, 5, 6) the analyzed vitamin E content of the diet increased to 124 IU/kg. And when vitamin E was supplemented at the higher level of 100 IU/kg (Treatments 7, 8, 9) the analyzed vitamin E content of the diet increased to an average of 128 IU/kg. Our analysis showed that basal level of Se was 0.2 ppm, increasing to 0.37 and 0.57 ppm when 0.25 and 0.50 SS was supplemented.

There were no treatment effects on feed intake ($P = 0.3656$), egg production ($P = 0.9761$) and hen weight gain ($P = 0.8155$) (Table 3). Table 4 shows the lack of treatment effect on egg weight ($P = 0.7194$), Haugh units ($P = 0.7076$), specific gravity ($P = 0.9908$), and fresh ($P = 0.1903$) and aged ($P = 0.1709$) albumen pH.

Fresh yolk pH was significantly lower ($P = 0.0121$) when hens were fed the basal level of SS (6.027) compared to 0.25 and 0.5 ppm Se supplementation (6.059 and 6.061, respectively) (Figure 1). When 50 IU/kg vitamin E was added to the diet, the eggs produced from this diet and aged for two weeks in the cooler at 7° C had significantly lower yolk pH of 6.180 ($P = 0.0083$) compared to 6.236 and 6.208 (from the diets supplemented with 0 or 100 IU/kg vitamin E, respectively) (Figure 2). Table 4 shows that there was a vitamin E by Se levels interaction that was approaching significance affecting aged yolk pH. When comparing fresh to aged yolk and albumen pH (Figures 3 & 4), it was observed that pH increased with aging.

As vitamin E increased in the diet, yolk α-tocopherol content increased significantly ($P < 0.0001$) (Figure 5); at 0 IU/kg vitamin E, yolk α-tocopherol was 44.07 µg/g, at 50 IU/kg yolk α-tocopherol was 192.13 µg/g, and at 100 IU/kg α-tocopherol content of yolk was 273.62 µga/g.

As vitamin E increased in the diet, yolk Se content decreased significantly ($P = 0.0034$), and with increasing SS level in the diet, yolk Se content increased significantly as well ($P < 0.0001$) (Table 5). Supplementation of 0.5 ppm sodium selenite with any vitamin E combination (0, 50, 100 IU/kg) always had a higher yolk Se content of 1213.75, 1088.75, and 1169.00 ppb, respectively ($P = 0.0058$). There was a significant interaction effect ($P = 0.0058$) between vitamin E and Se levels affecting yolk Se content; when hens were supplemented with 0.5 ppm Se, yolk Se content decreased significantly when vitamin E was added at 50 IU/kg (Treatment 6) compared to the treatment with no vitamin E (Treatment 3) (1213.75 vs. 1088.75 ppb) (Figure 6). The same effect was noticed when hens were supplemented with 0 ppm Se; yolk Se content decreased

significantly when vitamin E was added at 50 IU/kg (Treatment 4) compared to the treatment with no vitamin E (Treatment 1) (958.50 vs. 808.50 ppb). Figure 6 also shows that there was a negative effect of vitamin E on yolk Se content when hens were supplemented with 0.25 ppm Se; yolk Se content decreased significantly when vitamin E was added at 100 IU/kg (Treatment 8) compared to the treatment with 50 IU/kg vitamin E (Treatment 5) (992.25 vs. 868.25 ppb). When looking at simple effects of treatments on yolk Se content, the three treatments that gave the highest yolk Se content were Treatments 3, 6, and 9 as shown in Figure 7.

DISCUSSION

In corn, the α-tocopherol content varies between 6.0 to 20.0 mg/kg (McDowell, 1989; and Lynch, 1996). Basal levels of α-tocopherol for this study were near zero, indicating a very poor availability in the raw ingredients used in this study. Vitamin E concentration in feedstuffs is affected by many factors, including its exposure to heat, light, alkali or oxidizing chemicals. These factors can explain some of the results of the analyzed vitamin E content in the diets of our experiment (Table 2); the experiment was conducted between late December of 2006 to June of 2007, but the diets were not analyzed for vitamin E until September of 2010. Diets were kept in the freezer at -20°C for more than 3 years and those factors could have affected the values attained in the analysis. Selenium level, on the other hand, varies between 0.01 to 1.00 ppm in corn and 0.06 to 1.00 ppm in soybean meal (Surai, 2006). These wide variations depend upon selenium content of the soil from different geographical locations. For example, yellow corn in Nebraska and S. Dakota has 0.38 ppm Se where in other states its average is

0.025 ppm. Selenium content in soybean meal is 0.1 ppm in the Midwestern U.S.A. but in Nebraska alone it is 0.54 ppm (Leeson and Summers, 2001). Basal calculated level of vitamin E in this study was 20 IU/kg, which is four times the requirement of vitamin E in laying hens as stated in the 1994 NRC (5 IU/kg), and vitamin E was supplemented at 50 and 100 IU/kg. Basal calculated level of Se in the study was 0.20 ppm, whereas the 1994 NRC recommendation for laying hens with 100 g/d feed intake is 0.06 ppm. Sodium selenite (SS) was supplemented at 0.25 and 0.50 ppm. These higher values of basal vitamin E and Se and supplementation levels well above the levels in NRC were the basis of this research in an attempt to improve egg quality by decreasing the negative effects of free radicals on egg quality.

Our results showed that supplementation of vitamin E and selenium to the hens did not further improve or had any adverse effect on feed intake, egg production, Haugh units, egg weight, specific gravity, and fresh or aged albumen pH. These results may have been due to that hens were housed under thermoneutral conditions with no strong environmental stressors. Some of these results agree with the results of Puthpongsiriporn et al. (2001), who found that supplementation of vitamin E during thermoneutral conditions had no effect on feed intake level. In a study done by Sahin et al. (2001), higher levels of vitamin E resulted in a higher feed intake, and higher dietary Se inclusions caused an improvement in feed intake. Gowdy (2004) found that male Ross broiler chickens had a sharp decrease in body weight with SS feeding at 10 and 15 ppm.

Supplementation of vitamin E and SS above NRC (NRC, 1994) requirements did significantly affect some aspects of egg quality measurements. When Se was supplemented, fresh yolk pH increased, which was not expected. Whereas higher vitamin

E in the diet decreased aged yolk pH, indicating overall oxidation in the yolk was lowered with the addition of vitamin E. The increased pH in aged yolk and albumen compared to fresh yolk and albumen is a result of the dissociation of 2 proteins in the albumen (lysozyme and ovomucin) and carbon dioxide is being lost from the egg, which in turn reduces the viscosity of the albumen and the Haugh units (Williams, 1992; Brake et al., 1997).

The results of increased yolk α-tocopherol with higher vitamin E in the diet were expected as many previous studies have reported a linear relationship between vitamin E level in the diet and α- tocopherol in egg yolk (Kirunda et al., 2001; and Puthpongsiriporn et al., 2001). The reason behind the increase in yolk α-tocopherol as vitamin E increased in the diet is the direct transport of tocopherols accumulated in the liver to egg yolk as compounds of plasma Very-low-density lipoprotein (VLDL) (Cherian et al., 1996). Also yolk α-tocopherol content was analyzed at the end of the trial in summer 2007 when all the samples were still fresh and there was no storage or oxidation effect on them as was the case with the diet samples.

Negative interaction effects of supplemental vitamin E and Se on egg yolk deposition have not been previously reported. These findings are contrary to the sparing effect of vitamin E to selenium: as vitamin E increases in the diet it is thought to decrease Se-containing glutathione peroxidase, thus decreasing the Se requirement in other body tissues and increasing its deposition in the yolk (Leeson and Summers, 2001). However, some of our results agree with this; supplementation with 100 IU/kg vitamin E gave higher yolk Se content when there was no Se supplemented in the diet compared to 50 IU/kg, this can be explained by the less Se provided from the basal diet for antioxidation

in the egg, thus higher Se deposition in the yolk. The same trend was observed when hens were supplemented with 0.50 ppm Se; the higher yolk Se content was observed when hens were supplemented with 100 IU/kg compared to 50 IU/kg. When hens were supplemented with 0.25 ppm Se, yolk Se content also increased with increasing vitamin E content to 50 IU/kg compared to 0 IU/kg vitamin E.

Typically, an increase in vitamin E and Se supplementation will add some extra costs to the feed, but these extras are not very high and an improvement in the quality variables of eggs produced, which includes the higher yolk vitamin E and Se content, can easily cover the extra cost. The price of vitamin E from International Nutrition as of Dec 2010 with 20,000 IU/lb concentration was 80 cents/lb, and the sodium selenite with a 0. 02% concentration was 14 cents/lb, which added up to $3.642/ton of feed or 0.622 cents/dozen eggs for the maximum inclusion levels of 100 IU/kg of vitamin E and 0.5 ppm Se. Ordinary eggs are priced at around $1.80/dozen and the 'designer' eggs are usually around $3.50/dozen which is almost double the price of the ordinary ones, so supplementation of vitamin E and Se adds to the value of 'designer' eggs economically and adds nutritional value. Vitamin E and Se increased nutritional value of eggs providing up to 30% of the human RDA for Se requirement and 35% of the human RDA for vitamin E requirement (Fisinin et al., 2009; and Traber, 2003). One egg from our study at the highest levels of supplementation provided 22 µg Se (at 0.50 ppm) and a total of 5 IU α-tocopherol (at 100 IU/kg).

In conclusion, levels of dietary vitamin E and Se high above the NRC requirements did not negatively affect most of the egg production or quality parameters, but higher levels of vitamin E decreased the negative effects of egg storage on yolk

quality and produced higher yolk α-tocopherol content. Yolk Se content increased as a result of increasing Se level in the diet as well. The higher levels of vitamin E and Se in the laying hen diets could provide around 30% of the RDA of vitamin E or Se for human consumption.

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Table 2.1 Diet composition and nutrient content of the basal corn-soybean meal diet.

Ingredients	(Phase I) (%)		(Phase II) (%)	
Fine Ground Corn	63.29		61.29	
Soybean Meal- 47%	25.00		25.11	
Tallow	0.47		2.15	
Corn Oil	0.46		-	
PL Shell and Bone	-		4.71	
Limestone	8.52		4.71	
Dicalcium Phosphate	1.75		1.53	
NACL	0.32		0.40	
DL- Methionine	0.19		0.10	
Vitamin Premix[1]	0.20		0.20	
Mineral Premix[2]	0.10		0.10	
Nutrient Composition	**Calculated**	**Analyzed**	**Calculated**	**Analyzed**
ME, kcal/kg	2775	-	2820	-
Protein, %	16.70	16.42	16.50	16.11
Methionine, %	0.47	-	0.37	-
Met+Cys	0.75	-	0.65	-
Lysine, %	0.84	-	0.84	-
Ca, %	3.70	2.53	4.00	3.75
Total P, %	0.42	0.61	0.38	0.57
Alpha-tocopherol (IU/kg)	20.00	0.00	20.0	0.00
Se (ppm)	0.20	0.20	0.20	0.20
Sodium	0.15	-	0.18	-

[1] Provided per kilogram of diet: Vitamin A 29,964,000 IU; Vitamin D 35,200,000 IU; Vitamin K 35,200 mg; Vitamin B-12 1,320 mg; Riboflavin 798,336 mg; Niacin 498,960 mg; Pantothenic acid 323,855.4 mg; Folic acid 99,792 mg; Vitamin B-6 821,286.4 mg; Choline 598,400 mg; Thiamin 88,000 mg; Biotin 2,200 mg.

[2] Provided per kilogram of diet: Copper, 8.75 mg from copper sulfate; zinc, 35 mg from zinc sulfate; iodine, 0.035 mg from organic iodine; manganese, 20 mg from manganese sulfate; iron, 45 mg from iron sulfate.

Table 2.2 Dietary vitamin E and selenite combinations and analyzed vitamin E and Se content.

Treatment	Vitamin E (IU/kg)	Selenite (ppm)	Analyzed vitamin E content[3] (IU/kg)	Analyzed Se content[4] (ppb)
1	0	0.00	0	204.5
2	0	0.25	0	355.5
3	0	0.50	0	488.0
4	50	0.00	124	193.5
5	50	0.25	124	356.5
6	50	0.50	124	637.5
7	100	0.00	128	205.5
8	100	0.25	127	387.5
9	100	0.50	129	589.5

[3] Diet vitamin E was analyzed at Midwest Labs using Liquid Chromatographic Methods (A.O.A.C, 2007), Septemeber/2010.
Calculated basal level of vitamin E should have been around 20 mg/kg but due to the effects mentioned in discussion it came out to 0 IU/kg.

[4] Diet Se was analyzed at Alltech Labs using a PSA Millenium Excalibur system, which uses continuous flow vapour/hydride generation atomic fluorescene to analyze for selenium (Wallschlager et al., 2001), May/2009.

Table 2.3 Treatment effect on feed intake, egg production, and hen weight.

Treatment	Vitamin E (IU/kg)	Selenite (ppm)	Feed Intake (g/hen/d)	Egg Production (%)	Hen Weight Gain (g)
1	0	0.00	103.78	86.94	15.12
2	0	0.25	104.93	85.65	16.39
3	0	0.50	105.22	87.63	19.37
4	50	0.00	103.93	82.96	24.26
5	50	0.25	104.70	82.67	46.52
6	50	0.50	105.67	87.49	24.79
7	100	0.00	105.28	88.35	29.65
8	100	0.25	103.06	84.43	17.92
9	100	0.50	104.98	85.31	21.62
SEM[5]			0.9128	5.2750	15.33
Main Effects					
Vitamin E level (IU/kg)					
0			104.64	86.74	16.96
50			104.77	84.37	31.86
100			104.44	86.03	23.06
SEM			0.5509	2.5833	8.914
Selenite level (ppm)					
0.00			104.33	86.08	23.01
0.25			104.23	84.25	26.94
0.50			105.29	86.81	21.93
SEM			0.5505	2.5838	8.914
***P*-value**					
Vitamin E level			0.9034	0.8700	0.4922
Selenite level			0.2845	0.8488	0.9145
Vitamin E x Selenite level			0.3656	0.9761	0.8155

Means with no common superscripts differ significantly ($P \leq 0.05$).
[5] SEM: Standard Error of Mean.

Table 2.4 Treatment effect on egg weight, Haugh unit, specific gravity and yolk and albumen pH.

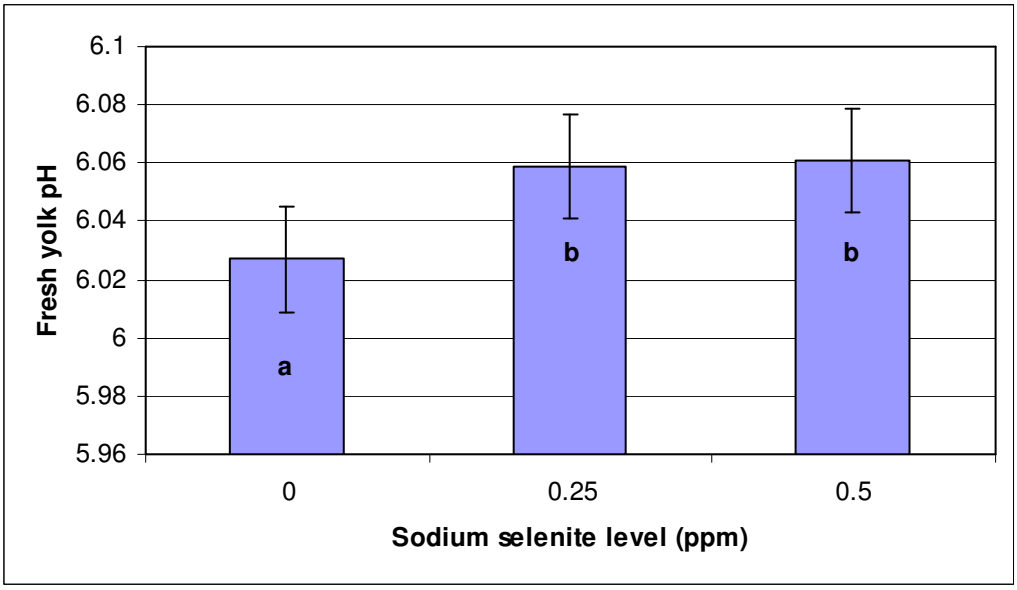
Treatment	Vitamin E (IU/kg)	Selenite (ppm)	Egg Weight (g)	Haugh Unit	Specific Gravity	Yolk pH		Albumen pH	
						Fresh	Aged	Fresh	Aged
1	0	0.00	60.45	92.77	1.082	6.024	6.259	8.453	9.100
2	0	0.25	59.96	91.82	1.080	6.066	6.181	8.544	9.122
3	0	0.50	59.32	93.19	1.088	6.060	6.269	8.564	9.097
4	50	0.00	59.19	91.98	1.080	6.038	6.201	8.596	9.125
5	50	0.25	59.92	92.45	1.081	6.042	6.177	8.503	9.113
6	50	0.50	59.78	92.25	1.087	6.037	6.163	8.555	9.111
7	100	0.00	60.62	91.87	1.086	6.019	6.197	8.540	9.129
8	100	0.25	61.03	92.57	1.082	6.071	6.206	8.543	9.073
9	100	0.50	59.98	91.80	1.090	6.086	6.221	8.542	9.102
SEM			0.70	0.88	0.70	0.021	0.022	0.046	0.013
Main Effects									
Vitamin E level (IU/kg)									
0			59.91	92.59	1.083	6.050	6.236[a]	8.520	9.106
50			59.63	92.23	1.083	6.039	6.180[b]	8.551	9.116
100			60.54	92.08	1.086	6.059	6.208[ab]	8.542	9.101
SEM			0.44	0.59	0.002	0.018	0.014	0.032	0.004
Selenite level (ppm)									
0.00			60.09	92.21	1.083	6.027[a]	6.219	8.529	9.118
0.25			60.30	92.28	1.081	6.059[b]	6.188	8.530	9.102
0.50			59.69	92.41	1.088	6.061[b]	6.217	8.554	9.103
SEM			0.44	0.59	0.002	0.018	0.014	0.032	0.003
***P*-value**									
Vitamin E level			0.242	0.7237	0.6623	0.2976	0.0083	0.6234	0.5051
Selenite level			0.5321	0.9498	0.1620	0.0121	0.1258	0.6867	0.392
Vitamin E x Selenite level			0.7104	0.7076	0.9908	0.2335	0.0779	0.1903	0.1709

Table 2.5 Treatment effect on yolk α-tocopherol and Se content.

Treatment	Vitamin E (IU/kg)	Selenite (ppm)	Yolk α-tocopherol (µg/g)	Yolk Se content (ppb)
1	0	0.00	46.75	958.50[a]
2	0	0.25	43.31	944.75[ab]
3	0	0.50	42.15	1213.75[d]
4	50	0.00	207.91	808.50[c]
5	50	0.25	186.69	992.25[a]
6	50	0.50	181.78	1088.75[e]
7	100	0.00	291.89	835.50[c]
8	100	0.25	331.77	868.25[cb]
9	100	0.50	197.08	1169.00[de]
	SEM		36.08	29.67
Main Effects				
Vitamin E level (IU/kg)				
0			44.07[a]	1039.00[a]
50			192.13[b]	963.17[b]
100			273.62[c]	957.58[b]
SEM			20.83	17.13
Selenite level (ppm)				
0.00			182.18	867.50[a]
0.25			187.25	935.08[b]
0.50			140.38	1157.17[c]
SEM			20.83	17.13
***P*-values**				
Vitamin E level			<0.0001	0.0034
Selenite level			0.2476	<0.0001
Vitamin E x Selenite level			0.3695	0.0058

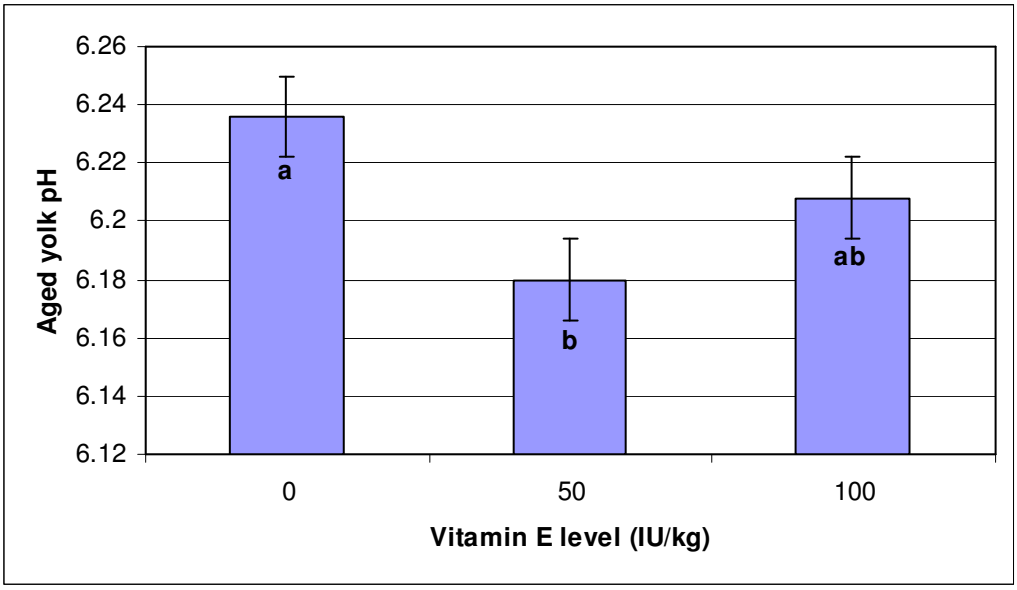
Means with no common superscripts differ significantly ($P \leq 0.05$).

Figure 2.1 Main effect of sodium selenite level on fresh yolk pH.



P = 0.0121

Figure 2.2 Main effect of vitamin E level on aged yolk pH.



P = 0.0083

Figure 2.3 Fresh vs. aged yolk pH.

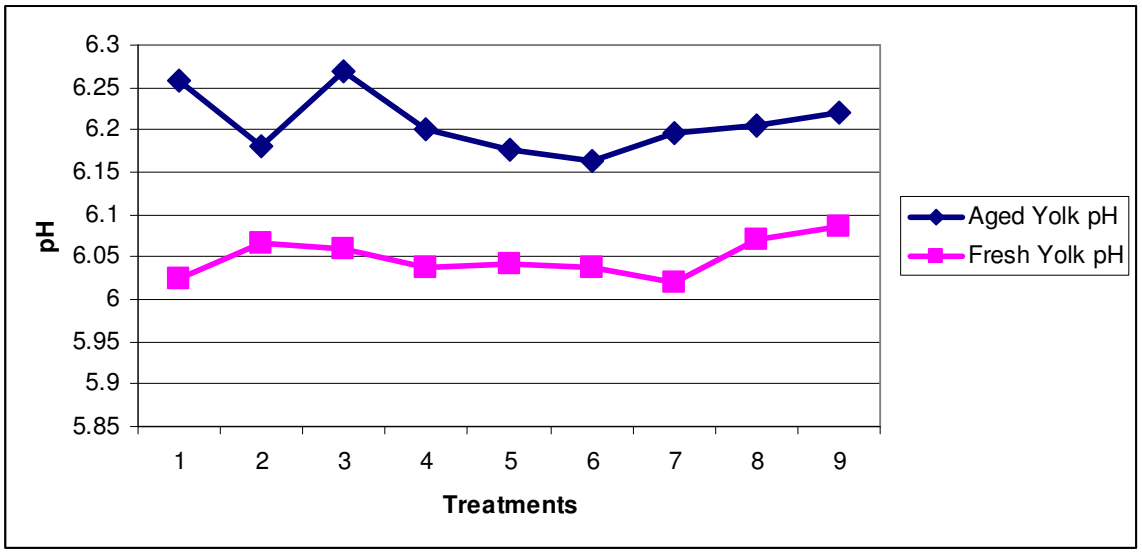


Figure 2.4 Fresh vs. aged albumen pH.

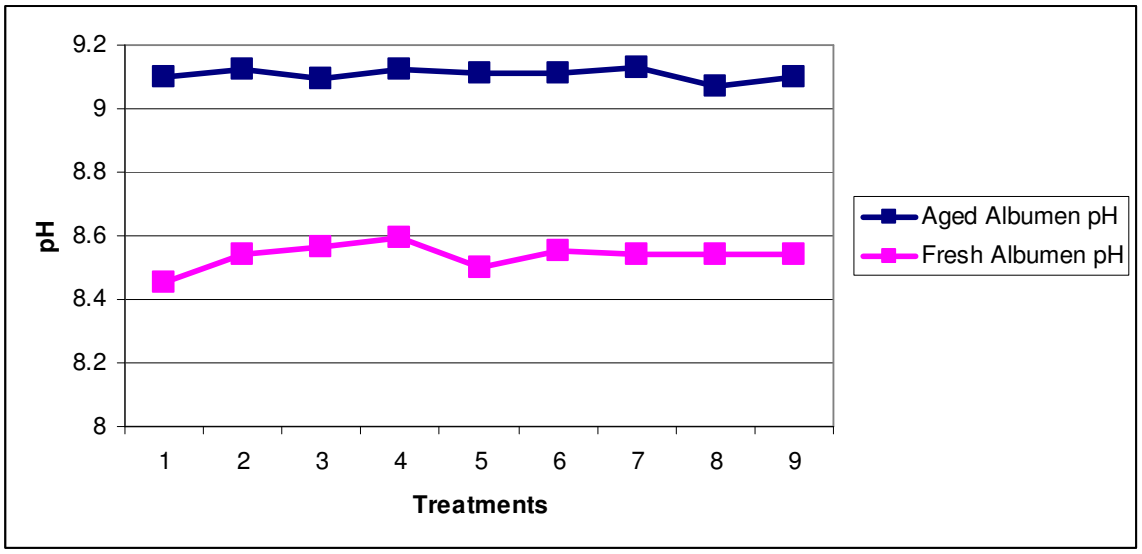
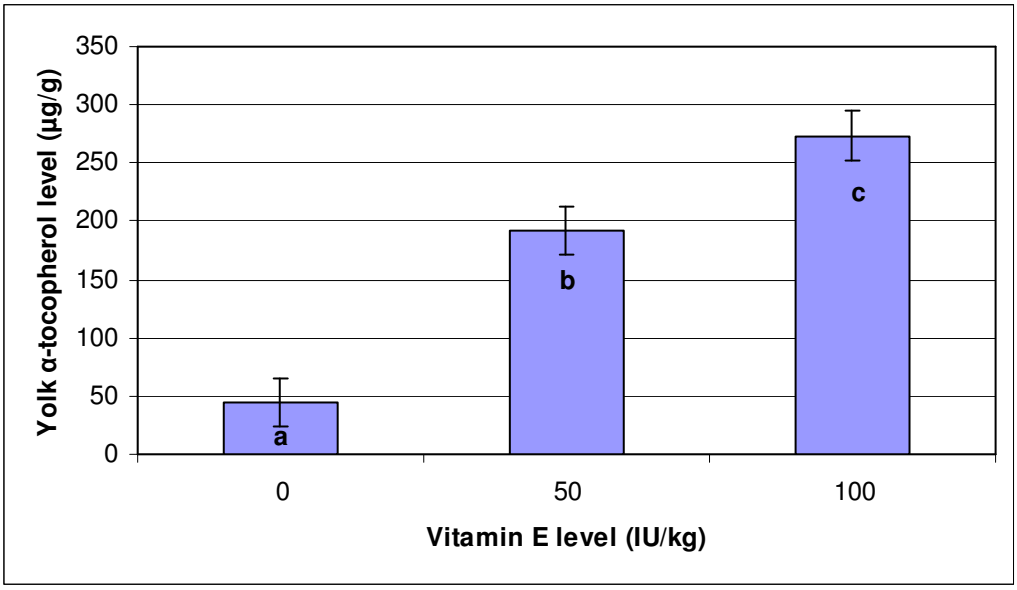
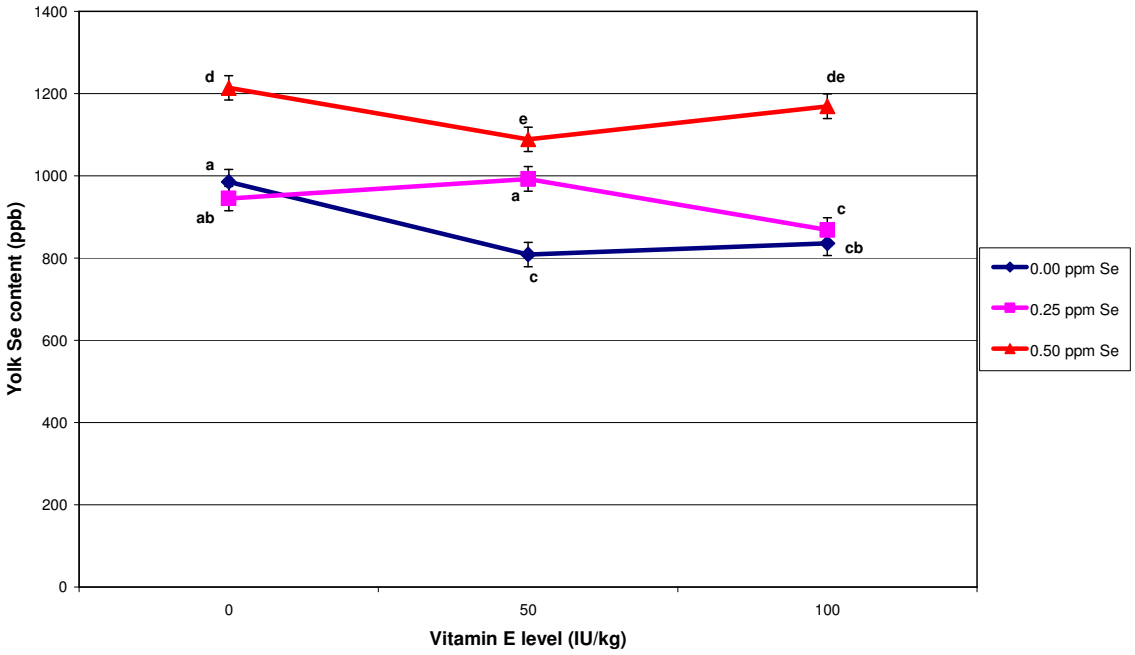


Figure 2.5 Main effect of vitamin E level on yolk α-tocopherol content.



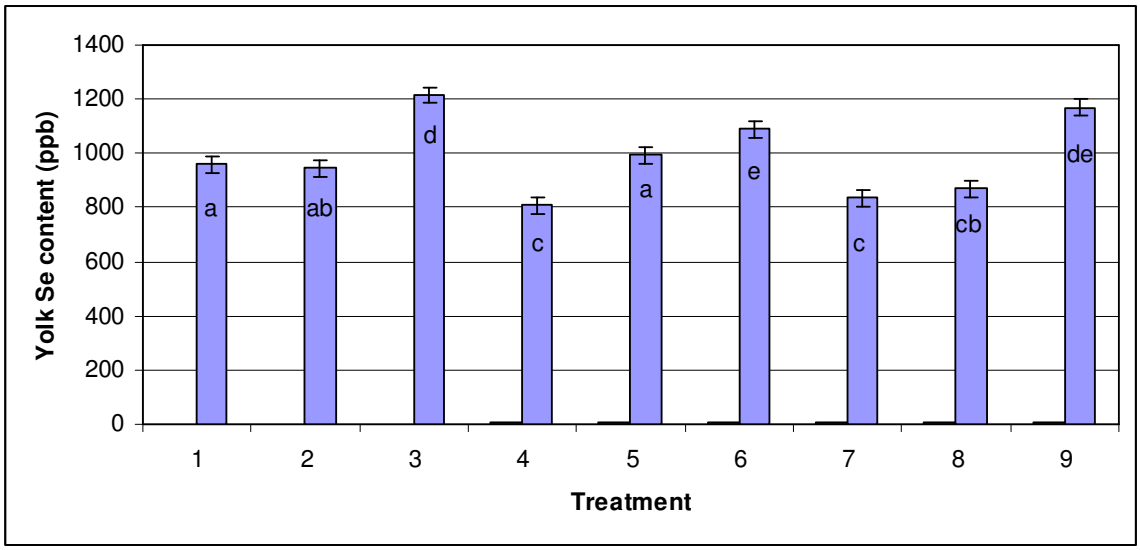
$P < 0.0001$

Figure 2.6 Interaction effect of vitamin E and sodium selenite levels on yolk selenium content.



$P = 0.0058$

Figure 2.7 Treatment effect on yolk Se content.



$P = 0.0058$

CHAPTER 3

The Effect of Selenomethionine vs. Sodium Selenite Supplementation on Vitelline Membrane Strength and Glutathione Peroxidase Activity in the Liver and Shell Gland of Laying Hens

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ABSTRACT The objective of this study was to investigate the effects of selenium (Se) source and level higher than NRC requirements on production parameters of laying hens, egg yolk vitelline membrane strength (VMS), and glutathione peroxidase (GSH-Px) activity in the liver and shell gland of hens. A total of 120 Hy-Line W36 White Leghorn laying hens were fed the experimental diets for 8 weeks. Hens were assigned to 30 cages with 6 cages/treatment. Cages were blocked by side, each side had a total of 15 cages, and tier, each tier had a total of 5 cages. Hens were fed a corn-soybean meal basal diet supplemented with 0.0, 0.2 ppm selenomethionine (SM), 0.2 ppm sodium selenite (SS), 0.4 ppm SM, or 0.4 ppm SS for a total of 5 dietary treatments in a factorial treatment design. The basal level of Se in the diet was 0.2 ppm. Feed intake and egg production were measured daily. Egg weight, specific gravity, and Haugh units were measured

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weekly. Fresh and aged yolk and albumen pH were measured biweekly. Vitelline membrane strength was measured at wk 5, 6, 7, and 8 of the trial. At the end of the study, 2 hens per cage were euthanized to measure GSH-Px activity of the liver and shell gland tissues. Feed intake ($P = 0.0182$) and egg production ($P = 0.0375$) increased as dietary Se supplementation increased in the diet. Dietary treatments had no significant effect on hen weight gain ($P = 0.4148$), egg weight ($P = 0.2058$), Haugh unit ($P = 0.3774$), specific gravity ($P = 0.4846$), fresh ($P = 0.1880$) and aged ($P = 0.4430$) yolk pH, or fresh albumen pH ($P = 0.8024$). Supplementing SS at 0.2 ppm or SM at 0.4 ppm had the same effect to improve the VMS ($P = 0.03$). Neither SM nor SS had any effect on GSH-Px activity in the liver ($P = 0.5123$) and shell gland ($P = 0.5305$) of hens. This research indicates that 0.4 ppm Se from SM significantly improved feed intake, egg production and VMS in eggs and using 0.2 ppm SS improved VMS as well.

Key words: selenium, laying hens, vitelline membrane strength, glutathione peroxidase, selenomethionine.

INTRODUCTION

Glutatione peroxidase (GSH-Px) is found in all body tissues where oxidative processes occur (Kohrle et al., 2000). It reduces hydrogen peroxides (H_2O_2) and other peroxides to water and alcohols, to prevent production of reactive oxygen species. Selenium was discovered as part of the enzyme GSH-Px in 1973 (Rotruck et al., 1973). Selenium, being a major component of GSH-Px, prevents cellular damage by free radicals produced as natural by-products of oxygen metabolism in the body, which can

affect the live performance and product quality of poultry (Surai, 2000). The activity of GSH-Px depends on Se supplementation in the diet, and the activity of GSH-Px in certain tissues could be used as an index of selenium adequacy (Paynter, 1979). In addition to its role in GSH-Px, selenium has been shown to slow the aging process, prevent muscle disorders, aid in the metabolism of the sulfur-containing amino acids, allow for normal fetal development during pregnancy, ensure proper function of the thyroid gland, and stimulate immune function (Moustafa et al., 2003).

The structural integrity of the vitelline membrane has been an increasingly important issue for the egg-breaking industry. The strength of the vitelline membrane is important to prevent contamination of egg yolk and albumen, and it can be affected by many factors including egg storage conditions, yolk and albumen pH, and Haugh units (Kirunda, and McKee, 2000). Improving egg quality characteristics by Se supplementation may subsequently improve vitelline membrane strength.

In accordance with the poultry NRC (1994) Se requirement is quite low; however, those data are not related to the commercial conditions in which poultry may confront different stresses that increase Se requirement to a much higher level than stated in NRC (0.06 ppm). Traditionally, Se has been added to poultry diets via inorganic sources, such as sodium selenite (Na_2SeO_3). Research has shown that organic Se is more bioavailable, safer, and better absorbed than Se in sodium selenite (Edens, 2002). A study done by Payne et al. (2005) indicated that organic Se from selenium yeast (selenomethionine) results in greater deposition of Se in eggs compared to sodium selenite. Scheideler et al. (2010) showed that selenium deposition in the egg yolk was significantly higher in eggs from hens supplemented with organic selenium source (selenomethioine) compared to

inorganic source (sodium selenite). These developments raise questions as to which form of Se is best for dietary supplementation to maximize egg quality.

Thus, the objective of this study was to evaluate different sources and levels of Se on egg production and quality parameters of laying hens, including vitelline membrane strength, and to evaluate the effect of treatments on GSH-Px activity in the liver and shell gland tissues when supplementation of Se is higher than NRC requirements.

MATERIALS AND METHODS

Birds and Housing

Hy-Line W36 White Leghorn pullet chicks were obtained from Hy-Line International[4] and were transported to the Animal Science Department, at the University of Nebraska-Lincoln, and kept there until the start of the trial. Animal care for the hens complied with procedures approved by the University of Nebraska Institutional Animal Care and Use Committee (IACUC) (Protocol # 06-10-044D).

A total of one-hundred-twenty, 35-wk-old White Leghorn laying hens were randomly assigned to 30 cages in a single laying hen unit with 4 hens per cage. Cages were blocked by side, north and south, each side with a total of 15 cages, and hens were given a corn-soybean meal basal diet supplemented with 0.0, 0.2 ppm selenomethionine (SM), 0.2 ppm sodium selenite (SS), 0.4 ppm SM, or 0.4 ppm SS for a total of 5 dietary treatments in a factorial treatment design. Selenomethionine (Sel-Plex) was provided by Alltech[5]. Each treatment was assigned to 6 replicate cages. Birds were fed the dietary

[4] Hy-line North America International, 1005 4th Ave Se, Spencer, IA, 51301-6002.
[5] Alltech: 3031 Catnip Hill Pike, Nicholasville, KY 40356, U.S.A.

treatments for 8 weeks from 35-42 weeks of age. Hens were maintained on a 16:8 hr light:dark cycle throughout the trial. Each bird had approximately 500 sq. cm. of cage floor space (Chore-Time cages)[6]. Water was supplied *ad libitum* by nipple drinkers and the cage unit was located in a windowless, ventilated room.

Diets

Diets (Table 1) were formulated to be isocaloric to provide 2874 kcal ME/kg of feed and isonitrogenous to provide 16.0 % crude protein (CP). Birds were provided with *ad libitum* access to feed (100-110 g of feed per hen per day) and water during the study.

The 5 different SS and SM combinations (Table 2) were added to the diets to meet the National Research Council (1994) nutrient requirements for laying hens. Selenium from both sources was weighed, along with the other minerals, and 5 premixes were mixed separately and then added to 150 pounds of feed in each formulation and mixed again for 10-15 minutes. Dietary samples were collected from each diet formulation. Two preparations were made, at the start of the trial and after 4 weeks. Dietary samples were stored at -20°C until chemical analysis was performed.

Measurements

Hen and Egg Parameters. Data collected included percent daily hen egg production and daily feed intake. Both egg production and feed intake were calculated on a hen/day basis. One days' total egg production was used to measure egg weight weekly and two eggs with similar weight were picked for measurement of Haugh units (Haugh, 1937),

[6] Chore-Time Poultry Production Systems: A division of CTB, Inc., 410 N Higbee street, P. O. Box 2000, Milford, IN, 46542-2000, U.S.A.

which was also done on a weekly basis. Egg weight and Haugh units were measured using Technical Services and Supplies (TSS) eggware[7]. Specific gravity was analyzed every week by placing all collected eggs from 1 days' egg production in graded sodium choloride (NaCl) solutions (1.070, 1.075, 1.080, 1.085, 1.090, 1.095 and 1.100). Hen weight was measured as a cage group on a monthly basis, and average hen weight was calculated by cage.

Egg Yolk and Albumen pH. Yolk and albumen pH were measured once every 2 wk on fresh eggs and on eggs aged for two weeks in a cooler at 7° C. Two eggs per cage were collected for pH measurements. The yolk was separated from albumen using an egg separator. Yolk and albumen were poured into different glass beakers and homogenized with a stirring bar, and then the sensing bulb of a glass pH electrode connected to a pH meter was used to measure the pH.

Egg Yolk Vitelline Membrane Strength. A texture analyzer machine (TA.XTPlus)[8] equipped with a 20-kg tension load cell and a crosshead speed of 10 mm/sec, was used to measure vitelline membrane strength of fresh eggs (Tharrington et al., 1998). A one millimeter width, rounded end, stainless steel probe was used to apply direct pressure to the membrane. Two eggs per cage were used for this measurement during weeks 5, 6, 7, and 8 of the trial. Eggs were broken, and yolks were separated from albumen and placed into a shallow dish. The height between the probe and base was set at 25-30 mm, and the calibration weight was set at 2 kg. Once the start button was pushed, the probe moved

[7] Technical Services and Supplies (TSS): York, England.
[8] Texture analyzer and exponent software from Texture Technologies Corp.: Ramona, CA, 25133 Hereford Dr. U.S.A.

towards the yolk to penetrate through the yolk vitelline membrane in the equatorial region. Care was taken to avoid contact with the germinal disc or the chalazae. The force it took the probe to penetrate through the vitelline membrane strength was then measured directly in grams.

Glutathione Peroxidase Determinations. Two hens per cage were euthanized at the end of the study, 1-2 g of the liver and shell gland cells were excised and immediately frozen in liquid Nitrogen and stored at -80°C until further use. The Calbiochem[9] Glutathione Peroxidase Assay Kit was used to measure the glutathione peroxidase (GPx) activity indirectly by a coupled reaction with glutathione reductase (GR). Oxidized glutathione (GSSG), produced upon reduction of hydroperoxide by glutathione perxidase is recycled to its reduced state by glutathione reductase and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm. Under conditions in which the glutathione peroxidase activity is rate limiting, the rate of decrease in A_{340} is directly proportional to the glutathione peroxidase activity in the sample (Ursini et al., 1985). Three samples were prepared for each run at each time; negative control, which was prepared by adding 155 µl assay buffer, 25 µl co-substrate mixture, and 10 µl cumene hydroperoxide to three plate wells; positive control, which was prepared by adding 135 µl assay buffer, 20 µl glutathione peroxidase, 25 µl co-substrate mixture, and 10 µl cumene hydroperoxide to three plate wells; and the diluted tissues, which were prepared by adding 145 µl assay buffer, 10 µl of the samples in the cryovials after thawing and vortexing, 25 µl co-substrate buffer, and 10 µl cumene hydroperoxide to the

[9] Calbiochem is a registered trademark of EMD Chemicals Inc. EMD Chemicals Inc. is the North American affilate of Merck KGaA, Darmstadt, Germany, 2010.

rest of the plate wells. The plate was shaken for a few seconds, and the absorbance was read on a microplate reader[10] once every minute at 340 nm to obtain 5 time points. To calculate the final results, change in absorbance (A_{340}) per min was measured by plotting the absorbance values as a function of time to obtain the slope (rate) of the linear portion of the curve, and two standard points on the linear portion of the curve were selected to determine the change in absorbance during that time using the equation

ΔA_{340}/min. = [A_{340} (Time 2)- A_{340} (Time 1)] / Time 2 (min.)- Time 1 (min.)

Glutathione peroxidase activity (nmol/min/ml) was calculated using the equation

GPx Activity = {[ΔA_{340}/min.] / 0.00373 μM^{-1}} * [0.19 ml/ 0.02 ml] * [Sample dilution]

Chemical Analysis. Dietary samples were collected from each feed mixing and were subsequently ground using a 1-mm screen Tecator cyclotec grinder[11]. All diets were analyzed for Ca (927.02), P (965.17), and crude protein (Kjeldahl Method) (988.05) (A.O.A.C., 1984). Selenium analysis in the diets was analyzed by Alltech[5] using a PSA Millenium Excalibur system[12], which uses continuous flow vapour/hydride generation atomic fluorescence to analyze for selenium (Wallschlager et. al, 2001). All dietary samples were analyzed in duplicate. The 2 batches of all dietary samples were analyzed separately and the results were averaged.

Statistical Analysis. All data were analyzed as repeated measure using GLIMMIX procedure of SAS (SAS 9.2, 2008). The experimental design was a repeated measures, randomized complete block design. Blocking was implemented in order to reduce the

[10] Microplate Reader: Synergy HT, Biotek, Inc.
[11] Tecator Cycotec Grinder: 1093 Sample Mill, Tecator, Hoganas, Sweden.
[12] P S Analytical: Arthur House, Crayfields Industrial Estate, Main Rd, Orpington, Kent, BR5 3HP, UK.

effect of temperature variation in the cage unit. Blocks were considered a random effect, dietary treatments were considered fixed. A total of five dietary treatments were given for hens in the experiment (0, 0.2 ppm SM, 0.2 ppm SS, 0.4 ppm SM, or 0.4 ppm SS) in a factorial treatment design. Cages were the experimental units, with 4 hens per cage, and 6 cages per treatment. Average values for the variables were generated and subsequently analyzed separately to determine differences between combinations of treatments. The following model was used to determine differences between treatments groups

$$Y_{ijklm} = \mu + R_{k+} \alpha_i + \beta_j + \alpha\beta_{ij} + \tau_l + \alpha\tau_{il} + \beta\tau_{jl} + \alpha\beta\tau_{ijl} + \varepsilon_{ijklm}$$

Where

Y_{ijklm} = Variable measured.

μ = Overall mean.

R_l = Effect of l^{th} block.

α_i = Selenium level effect.

β_j = Selenium source effect.

$\alpha\beta_{ij}$ = Interaction effect of Se level and source.

τ_l = Time effect.

$\alpha\tau_{il}$ = Interaction effect of Se level and time.

$\beta\tau_{jl}$ = Interaction effect of Se source and time.

$\alpha\beta\tau_{ijl}$ = Intreraction effect of Se source and level and time.

ε_{ijklm} = Residual error.

The separation of means was done using LS means statement with pdiff option.

The data for Glutathione peroxidase activity in the liver and shell gland shells were analyzed using the model

$Y_{ijkl} = \mu + R_{k+} \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijkl}$

Where

Y_{ijkl} = Variable measured.

μ = Overall mean.

R_k = Effect of k^{th} block.

α_i = Selenium level effect.

β_j = Selenium source effect.

$\alpha\beta_{ij}$ = Interaction effect of Se level and source.

ε_{ijkl} = Residual error.

The separation of means was done using LS means statement with pdiff option.

RESULTS

Table 1 shows the diet composition and nutrient content of the standard corn-soybean meal basal diet and the analyzed protein, Ca, and P. The diet provided 2874 kcal/kg ME and 16.00% protein. Table 2 shows the dietary sodium selenite (SS) and selenomethionine (SM) treatment combinations and analyzed total dietary Se content.

When looking at the main effect of dietary Se level (Table 3), feed intake increased as Se supplementation increased in the diet from 87.20 at 0.2 ppm Se supplementation to 90.27 g/hen/d at 0.4 ppm Se supplementation in the diet ($P = 0.0182$). There was a Se source X Se level interaction affecting feed intake ($P = 0.0303$) (Figure 1). Simple effects of treatments on feed intake showed that both Se sources at 0.4 ppm supplementation and 0.2 ppm SS gave higher feed intake than the other 2 treatments (Treatments 1 and 2) ($P = 0.0075$) with intakes reaching 89.68, 90.56, and 89.98 g/hen/d

for Treatments 3, 4, and 5, respectively, compared to 86.10 and 84.73 g/hen/d for Treatments 1 and 2, respectively (Figure 2).

Selenium source had a significant effect on egg production, with hens fed SS having higher egg production than hens fed SM (83.44 vs. 79.69%) ($P = 0.0095$). When looking at Se level, supplementation with 0.4 ppm Se gave a significantly higher egg production of 82.99% than 0.2 ppm Se of 80.15% ($P = 0.0375$) (Table 3). There was a Se source X Se level interaction effect on egg production ($P = 0.0039$). Egg production was higher in the control group and the groups supplemented with 0.2 ppm SS or 0.4 ppm from either source than the group supplemented with 0.2 ppm SM ($P < 0.0039$) (Figure 3). Figure 4 shows the treatment effect on egg production ($P < 0.001$). The same trend was observed in egg production as feed intake; higher egg production percentages were attained with Treatments 3, 4, and 5 than Treatments 1 and 2, although Treatment 5 was not significantly higher than Treatment 1.

Dietary treatments had no effect on hen weight gain ($P = 0.4148$), egg weight ($P = 0.2058$), Haugh unit ($P = 0.3774$), or specific gravity ($P = 0.4846$) (Tables 3 and 4). As SM increased in the diet from 0.2 to 0.4 ppm, vitelline membrane strength (VMS) increased (6.44 vs. 6.75 g) ($P = 0.0064$), but when using SS as the source of Se, VMS had the tendency to decrease with increasing SS in the diet (Figure 5). When comparing treatment effect on VMS, Treatment 4, which was supplemented with 0.4 ppm SM, gave the highest VMS (Figure 6).

From Table 4, there was a Se source effect on aged albumen pH approaching significance ($P = 0.0563$); SM gave lower aged albumen pH than SS (9.268 vs. 9.280).

There was also a Se source X Se level effect on aged albumen pH approaching significance ($P = 0.0563$).

Neither Se level nor source had an effect on GSH-Px activity in the liver ($P = 0.5123$) or shell gland ($P = 0.5305$) of hens in this study (Table 5).

DISCUSSION

Our analysis shows that the non-supplemented diets had an average of 234.3 ppb Se and this level increased as the supplemental Se level increased in the diet (Table 2). Supplementation with higher levels of Se in the diet resulted in higher values of the total analyzed Se content, but this increase was more obvious when using selenomethionine (SM) in the diet rather than sodium selenite (SS). Whether this result was a consequence of a mixing error or less availability of SS than SM is not known. Selenium content in grains depends on Se level in the soils the plants were grown in. For example, researchers show that soils in the high plains of northern Nebraska and the Dakotas have very high levels of selenium, and people living in those regions generally have the highest selenium intakes in the United States (Longnecker et al., 1991). Selenium level varies between 0.01 and 1.00 ppm in corn, whereas in soybean meal it varies between 0.06 and 1.00 ppm depending on the soils that they were grown in (Surai, 2006).

Previously, Paton et al. (2002) found no significant difference in feed intake of hens supplemented with 0.0, 0.1, 0.2, or 0.3 ppm organic or inorganic Se which disagrees with the findings in our results. Cantor and Scott (1974) reported an increase in egg production of hens fed 0.1 ppm of organic selenium relative to no supplementation. Our results also disagree with those of Cantor et al. (2000) and Paton (2000) who reported no

difference in egg production when hens were fed a basal diet supplemented with 0.3 ppm of SS or the organic source (selenium-enriched yeast). Some of our results could be explained by the feed intake results in this study; supplementation with SS resulted in higher feed intakes which could have affected egg production from hens supplemented with SS compared to SM ($P = 0.0095$). In addition, adding 0.4 ppm Se significantly increased feed intake in hens and as a result egg production increased as well ($P = 0.0375$).

The lack of results on hen weight gain, egg weight, Haugh units, and specific gravity maybe explained by the fact that the basal diet had a high level of Se (234.25 ppb), and that further increases in supplemental Se level showed no further positive effects on these variables. In experiments conducted in Japan (Wakebe, 1999), Haugh units were used as an indicator of egg freshness, the authors reported the value was high on day 1 in both the control diet and the diet supplemented with 0.3 ppm Se. As time progressed, Haugh units of the control group declined sharply while the decline was more moderate in the treatment group. By day 7, it was clear that the Haugh units were significantly higher in the treatment group. The effect of Se on the interior quality of eggs can depend on age of the hens, composition of the diet, and conditions of egg storage (Paton and Cantor, 2000). Paton and Cantor (2000) were not able to show an effect of dietary Se source on egg Haugh units.

Scheideler et al. (2010) studied the effects of Se supplementation from SS and SM at two dietary levels (0.55 or 0.75 ppm) in laying hen diets and their effects on egg production and vitelline membrane strength (VMS). Increasing dietary selenium improved VMS in both fresh and aged eggs in Scheideler's study, and this agrees with

the findings of our study. Schafer et al. (1998) found that the protein percentage of the whole VM was on average about 70%; thus, knowing that Se is incorporated into proteins in the body, Se supplementation to the laying hen diets may change the composition of the proteins in the VM and contribute to its strength.

The tendency to decrease aged albumen pH with SM supplementation is a positive effect indicating less deterioration of egg proteins during storage. The increased pH in aged vs. fresh albumen (Table 4) was likely a result of a dissociation of two of the albumen's proteins (lysozyme and ovomucin), which in turn reduces viscosity of the albumen and egg quality (Powrie, 1977). The pH of a newly laid egg is between 7.6 and 8.5 (Heath, 1977); however, during storage the pH increases at a temperature-dependent rate to about pH 9.7, and this is due to CO_2 diffusion out of the egg (Sharp and Powell, 1931). Heath (1977) observed that the sulfhydryl content of the egg increases with increasing pH as the egg ages and that is associated with albumen thinning as a result of the uncoiling of albumen proteins.

The antioxidant properties of various selenoproteins are important to maintain antioxidant protection of the oviduct during egg shell formation (Surai, 2002) and for the protection of liver cells during yolk formation. The potential reason for a lack of dietary treatment effect on enzyme activities may have been due to the basal diet containing a level of 234.25 ppb Se that was already adequate and that further increases beyond that did not change the activity.

This study has demonstrated that addition of organic Se (Sel-Plex) improves some of the egg quality variables, particularly VMS and aged albumen pH at the highest level of Se inclusion of 0.4 ppm, which is higher than that approved by the FDA for Se (0.3

ppm). Thus, it is our opinion that the FDA should consider the permission of higher amounts of Se in the diet of laying hens, particularly selenomethionine.

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Table 3.1 Diet composition and nutrient content of the basal corn-soybean meal diet.

Ingredients	**% Diet**	
Fine Ground Corn	65.91	
Soybean Meal- 47%	20.53	
Tallow	1.81	
Limestone	4.49	
Dicalcium Phosphate	1.97	
Shell and Bone	4.49	
NaCl	0.37	
DL- Methionine	0.20	
Lysine	0.14	
Vitamin Premix[1]	0.20	
Mineral Premix[2]	0.10	
Composition	**Calculated Nutrient Composition**	**Analyzed Nutrient Composition**
ME, kcal/kg	2874	-
Protein, %	16.00	16.30
Methionine, %	0.45	-
Met+Cys	0.72	-
Lysine, %	0.88	-
Ca, %	3.94	3.57
Total P, %	0.73	0.66
Se (ppm)	0.20	0.20
Sodium	0.17	-

[1] Provided per kilogram of diet: Vitamin A 29,964,000 IU; Vitamin D 35,200,000 IU; Vitamin K 35,200 mg; Vitamin B-12 1,320 mg; Riboflavin 798,336 mg; Niacin 498,960 mg; Pantothenic acid 323,855.4 mg; Folic acid 99,792 mg; Vitamin B-6 821,286.4 mg; Vitamin E 88,000 IU; Choline 598,400 mg; Thiamin 88,000 mg; Biotin 2,200 mg.

[2] Provided per kilogram of diet: Copper, 8.75 mg from copper sulfate; zinc, 35 mg from zinc sulfate; iodine, 0.035 mg from organic iodine; manganese, 20 mg from manganese sulfate; iron, 45 mg from iron sulfate.

Table 3.2 Dietary Se source combinations and analyzed Se content.

Treatment	Source		Analyzed Se content
	Selenite (ppm)	**Selenomethionine** (ppm)	(ppb)
1 (Control)	0.0	0.0	234.25
2	0.0	0.2	356.00
3	0.2	0.0	314.50
4	0.0	0.4	470.75
5	0.4	0.0	334.25

[3] Diet Se was analyzed at Alltech Labs using a PSA Millenium Excalibur system, which uses continuous flow vapour/hydride generation atomic fluorescene to analyze for selenium (Wallschlager et al., 2001), Dec/2007.

Table 3.3 Treatment effect on feed intake, egg production, and hen weight gain.

Treatment	Source		Feed Intake	Egg Production	Hen Weight Gain
	Selenite (ppm)	Selenomethionine (ppm)	(g/hen/d)	(%)	(g)
1 (Control)	0.0	0.0	86.10[a]	80.23[a]	225.97
2	0.0	0.2	84.73[a]	76.70[b]	266.66
3	0.2	0.0	89.68[b]	84.56[c]	230.39
4	0.0	0.4	90.56[b]	83.21[c]	253.13
5	0.4	0.0	89.98[b]	82.70[ac]	279.47
***P* value**			0.0075	<0.0001	0.4148
SEM[4]			1.6601	1.0355	22.6040
Main Effects					
Se Source					
Selenomethionine			87.64	79.69[a]	258.13
Selenite			89.83	83.44[b]	253.76
SEM			1.1364	1.2173	25.0741
Se Level (ppm)					
0.2			87.20[a]	80.15[a]	246.56
0.4			90.27[b]	82.99[b]	265.33
SEM			1.1364	1.2173	25.0741
***P* values**					
Se Source			0.0761	0.0095	0.8644
Se Level			0.0182	0.0375	0.4685
Se Source x Se Level			0.0303	0.0039	0.2925
Control vs. Treatments 2, 3, 4, 5			0.0640	0.1441	0.2430

[a,b,c] Different superscripts within one column are significantly different at $P < 0.05$.

[4] SEM: Standard Error of Mean.

Table 3.4 Treatment effect on egg quality parameters.

Treatment	Source		Egg Weight	HU[5]	Specific Gravity	VMS[6]	Yolk pH		Albumen pH	
	Selenite	Selenomethionine					Fresh	Aged	Fresh	Aged
	(ppm)	(ppm)	(g)			(g)				
1 (Control)	0.0	0.0	56.41	98.83	1.087	6.52[ac]	6.155	6.406	8.435	9.260
2	0.0	0.2	56.50	98.17	1.087	6.44[c]	6.163	6.392	8.390	9.277
3	0.2	0.0	57.57	99.53	1.087	6.69[ab]	6.254	6.357	8.428	9.277
4	0.0	0.4	57.70	98.56	1.086	6.75[b]	6.190	6.299	8.396	9.259
5	0.4	0.0	57.67	97.60	1.093	6.52[ac]	6.152	6.315	8.455	9.284
***P* value**			0.2058	0.3774	0.4846	0.0335	0.1880	0.4430	0.8024	0.0577
SEM			0.5673	0.7094	0.0028	0.0730	0.0382	0.0480	0.0430	0.0073
Main Effects										
Se Source										
Selenomethionine			57.12	98.36	1.087	6.60	6.176	6.345	8.393	9.268
Selenite			57.41	98.36	1.091	6.60	6.203	6.336	8.441	9.280
SEM			0.4704	0.6413	0.0035	0.0747	0.0341	0.0418	0.0321	0.0059
Se Level (ppm)										
0.2			56.84	98.64	1.087	6.57	6.209	6.374	8.409	9.277
0.4			57.69	98.08	1.090	6.63	6.171	6.307	8.425	9.271
SEM			0.4685	0.6413	0.0035	0.0747	0.0341	0.0418	0.0321	0.0059
***P* values**										
Se Source			0.5515	0.9922	0.2784	0.9880	0.4446	0.8244	0.1497	0.0563
Se Level			0.0896	0.3951	0.3366	0.3972	0.2855	0.1274	0.6152	0.3393
Se Source x Se Level			0.5068	0.1537	0.2784	0.0064	0.0799	0.5590	0.7382	0.0563
Control vs. Treatments 2, 3, 4, 5			0.1121	0.6295	0.7045	0.3131	0.3505	0.2298	0.7179	0.0704

[a,b,c] Different superscripts within one column are significantly different at $P < 0.05$.

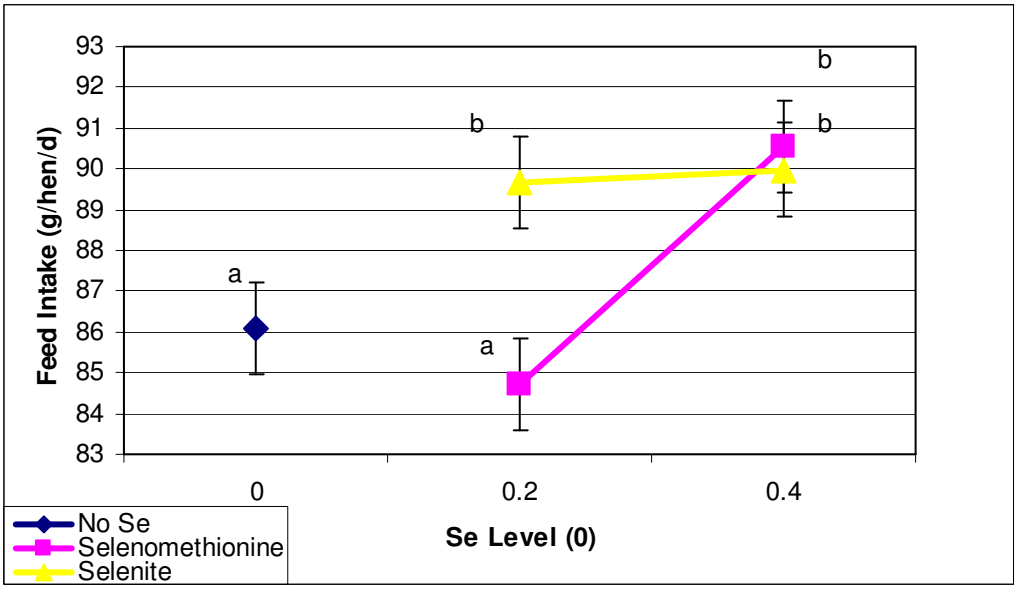
[5] HU: haugh unit.

[6] VMS: vitelline membrane strength.

Table 3.5 Treatment effect on liver and shell gland GSH-Px activity.

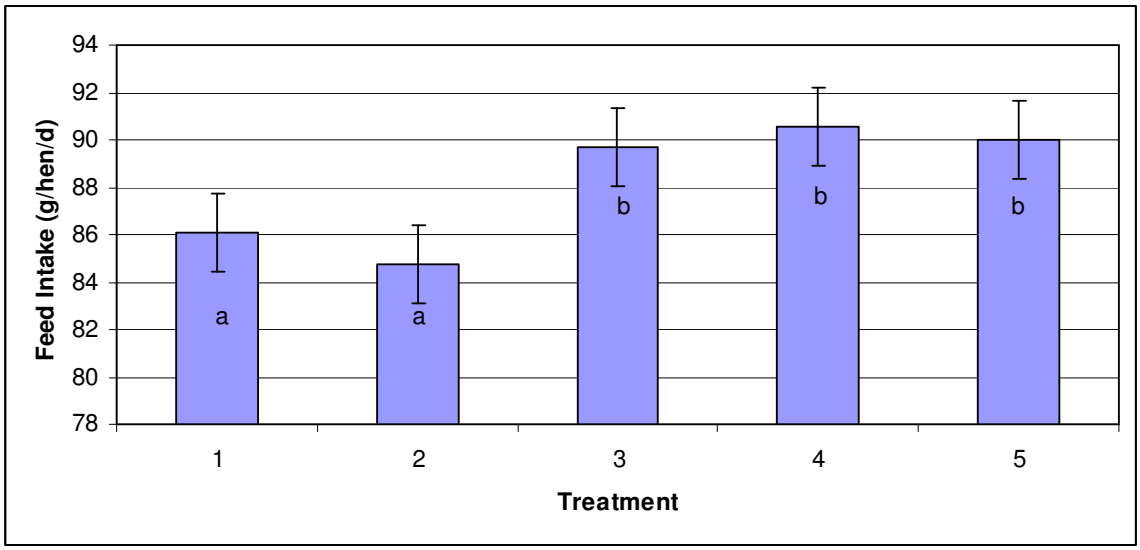
	Source		GSH-Px Activity	
Treatments	**Selenite**	**Selenomethionine**	**Liver**	**Shell Gland**
	(ppm)	(ppm)	(nmol/min/ml)	
1	0.0	0.0	261.68	211.67
2	0.0	0.2	250.23	102.72
3	0.2	0.0	148.97	86.44
4	0.0	0.4	191.44	116.34
5	0.4	0.0	158.59	108.26
***P* Value**			0.5123	0.5305
SEM			59.4752	56.0208
Main Effects				
Se Source				
Selenomethionine			219.55	108.67
Selenite			153.71	99.5646
SEM			54.5599	24.1001
Se Level (ppm)				
0.2			199.95	94.5808
0.4			173.31	113.65
SEM			26.6385	24.0946
***P* values**				
Se Source			0.2417	0.7097
Se Level			0.6307	0.4380
Se Source x Se Level			0.5154	0.7691
Control vs. Treatments 2, 3, 4, 5			0.2427	0.0923

Figure 3.1 Interaction effect of Se source and Se level on feed intake.



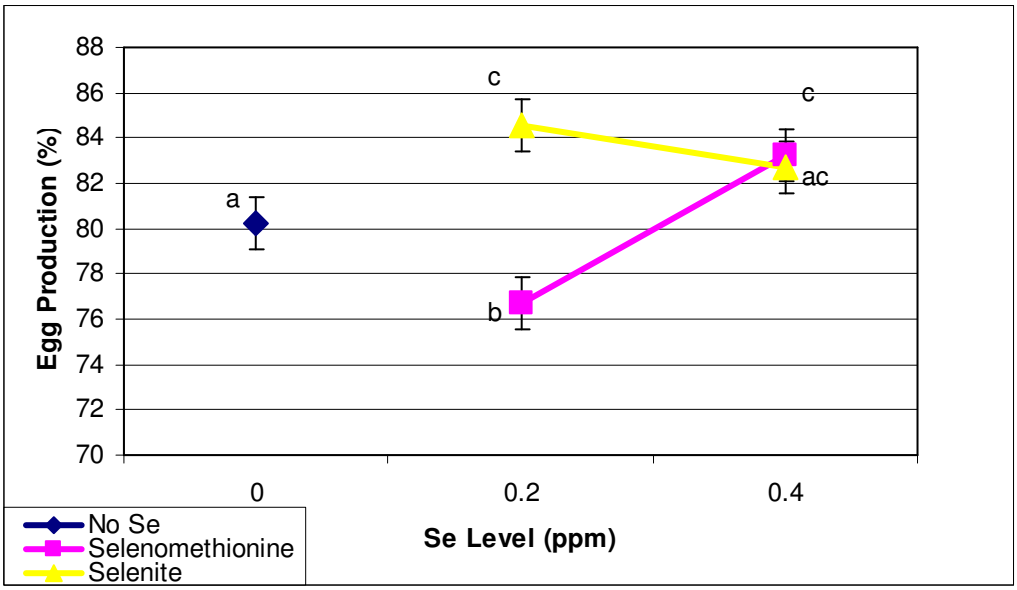
P = 0.0303

Figure 3.2 Treatment effect on feed intake.



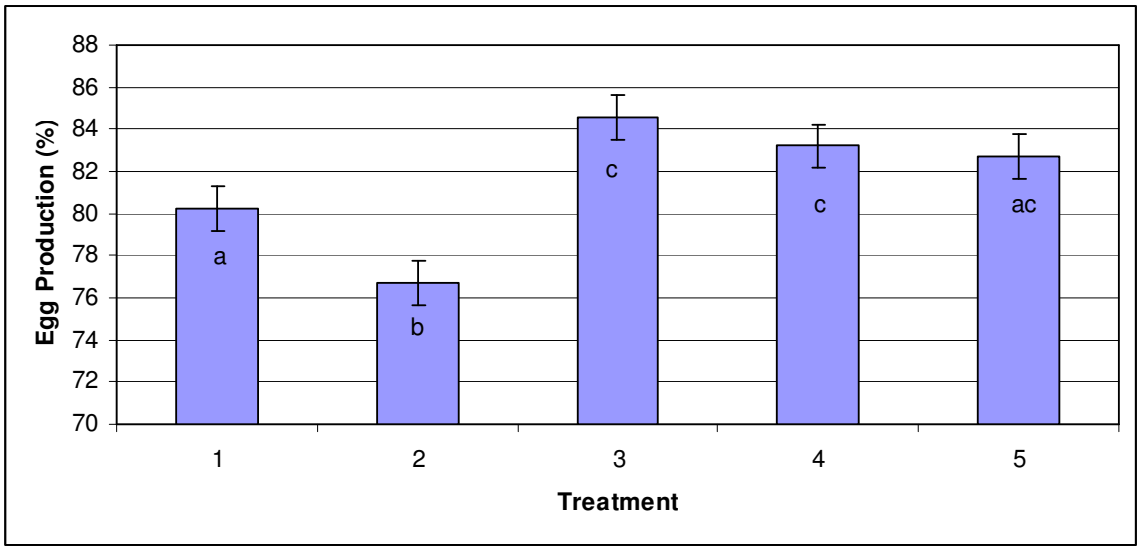
$P = 0.0075$

Figure 3.3 Interaction effect of Se source and Se level on egg production.



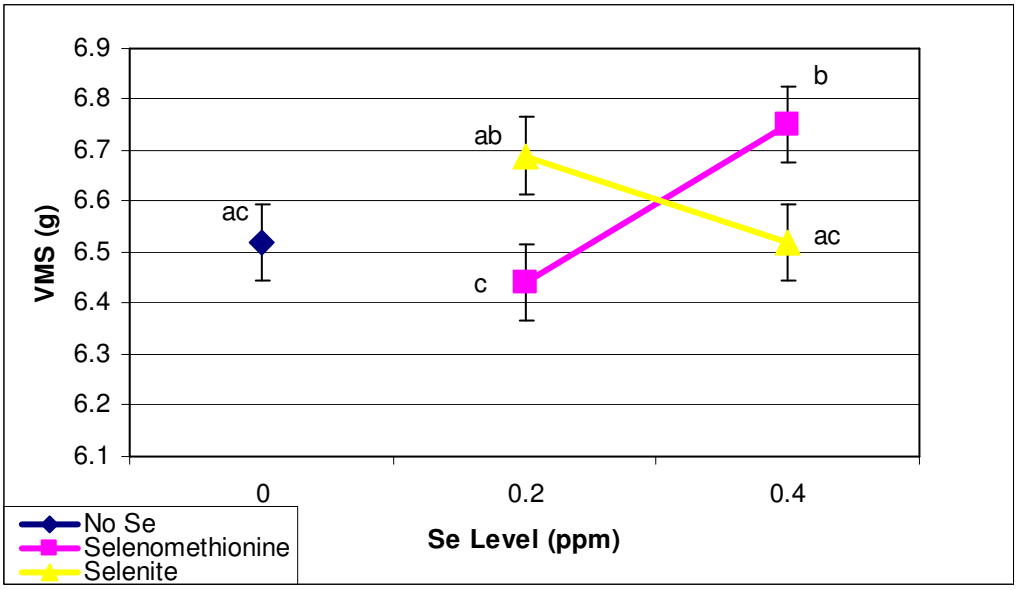
P = 0.0039

Figure 3.4 Treatment effect on egg production.



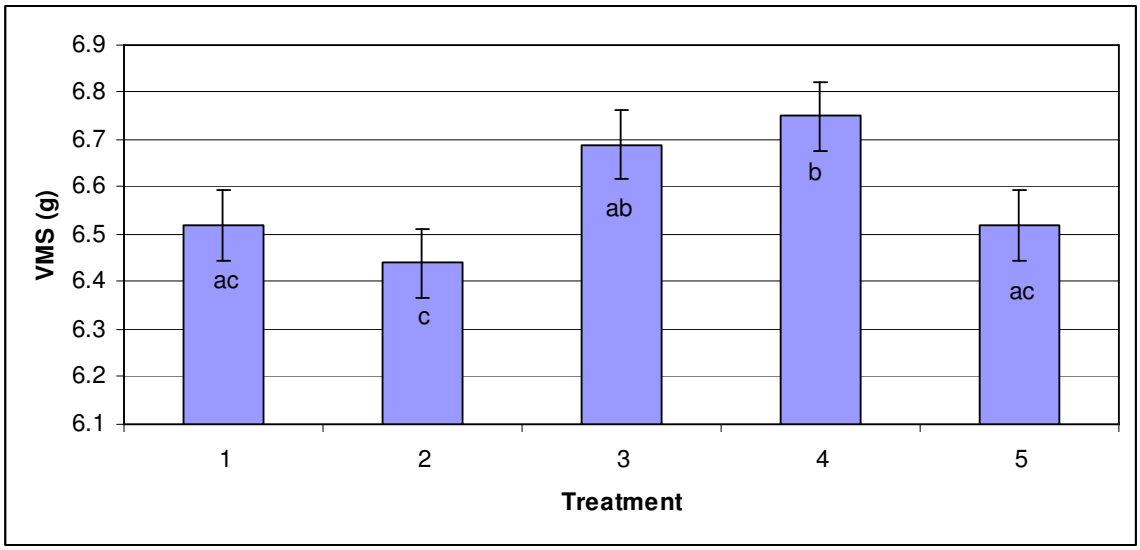
$P < 0.0001$

Figure 3.5 Interaction effect of Se source and Se level on VMS.



P = 0.0064

Figure 3.6 Treatment effect on VMS.



P = 0.0335

CHAPTER 4

The Effect of Selenomethionine vs. Sodium Selenite Supplementation on Vitelline Membrane Strength, Gutathione Peroxidase Activity in the Liver and Magnum of Laying Hens, and Egg Se Content When Using a Corn Starch Based Diet

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ABSTRACT The objective of this study was to investigate the effects of dietary selenium (Se) source and level on production parameters of laying hens fed a semi-purified diet. A total of 90 White Bovan hens were fed the experimental diets for 6 weeks. Hens were assigned to 30 cages with 6 replicate cages/treatment. Cages were blocked by side, north and south, each side with a total of 15 cages, and by side, each side with a total of 5 cages. Hens were fed a semi-purified corn starch-soybean meal diet supplemented, as calculated with: 0.0, 0.2 ppm selenomethionine (SM), 0.2 ppm sodium selenite (SS), 0.4 ppm SM, or 0.4 ppm SS, actual: 417.5, 483.83, 601.17, 822.83, or 591.33 ppm Se, respectively for a total of 5 dietary treatments in a factorial treatment arrangement. Actual Se levels in analyzed diet samples were higher than expected due to the basal diet having 417.5 ppm Se. Three eggs per treatment were collected three times throughout the study for yolk and albumen Se content. At the end of the study, 2 hens/

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cage were euthanized to measure GSH-Px activity of the liver and magnum tissues. Feed intake (P = (0.0034) and egg production (P = 0.0189) increased as dietary Se supplementation increased in the diet. Hens supplemented with selenomethionine consumed more feed than hens fed the inorganic source of Se ($P < 0.05$). Yolk Se content was higher in all treatments supplemented with Se from either source than the control diet (P = 0.0497) (1.57, 1.41. 1.63, 1.49 vs. 1.24 µg/g for Treatments 2, 3, 4, 5 vs. Treatment 1). There was a significant interaction effect of Se source and level on albumen Se content (P = 0.0358); albumen Se content increased from 2.33 to 2.93 µg/g when SM levels increased in the diet but not with increasing SS (1.90 to 1.95 µg/g). Dietary treatments had no effect on egg quality parameters or GSH-Px activity in the liver or magnum of hens. In conclusion, higher levels of Se that reached 0.8 ppm, as analyzed, did not have negative effects on egg quality; rather, it increased egg yolk Se content to a level that can contribute 2 times the human RDA (a total of 100 µg).

Key words: vitelline membrane strength, glutathione peroxidase, egg Se content.

INTRODUCTION

Selenium, as a major component of GSH-Px (Rotruck et al., 1973), prevents cellular damage by free radicals produced as natural by-products of oxygen metabolism in the body, which can affect the live performance and product quality of poultry (Surai, 2000). The activity of GSH-Px depends on Se supplementation in the diet, and the activity of GSH-Px in certain tissues could be used as an index of selenium adequacy (Paynter, 1979).

The strength of the vitelline membrane has been an increasingly important issue for the egg-breaking industry to prevent contamination of the albumen by the yolk. The strength of the vitelline membrane can be affected by egg storage conditions, yolk and albumen pH, and Haugh unit of eggs (Kirunda, and McKee, 2000). Improving egg quality characteristics by Se supplementation may improve vitelline membrane strength as a result (Scheideler et al., 2010).

Research has shown that organic Se has a higher availability than sodium selenite and it is safer, and readily absorbed (Edens, 2002). A study done by Payne et al. (2005) indicated that organic Se from selenium yeast (selenomethionine) results in greater deposition of Se in eggs compared to sodium selenite. Monsalve et al. (2004) showed that selenium deposition in the egg yolk was significantly higher in eggs from hens supplemented with organic selenium source compared to inorganic source.

Previous research has shown that corn-soybean meal basal diets used in laying hen rations are high in Se in Nebraska and S. Dakota, because of the high level of Se in corn (0.38 ppm) and soybean meal (0.54 ppm) (Leeson and Summers, 2001). To study the true effects of Se supplementation in laying hens, a basal diet low in Se was formulated and utilized. A semi-purified diet utilizing corn starch as an energy source should decrease basal diet Se level. Thus, the objective of this study was to evaluate different sources and levels of Se on egg production and quality parameters of laying hens using a semi-purified basal diet.

MATERIALS AND METHODS

Birds and Housing

A total of 90 Single Comb White Leghorn hens (Bovans White)[4], 30 weeks old, were obtained from a commercial laying hen operation, transported to the Animal Science Department, at the University of Nebraska-Lincoln, and kept there until the start of the trial. Animal care for the hens complied with procedures approved by the University of Nebraska Institutional Animal Care and Use Committee (IACUC) (Protocol # 06-10-044D).

Hens were randomly assigned to thirty cages in a single laying hen unit with three hens per cage. Cages were blocked by side, north and south, each side with a total of 15 cages, and by tier, each tier with a total of 5 cages, and hens were given a starch-soybean meal semi-purified diet supplemented with 0.0, 0.2 ppm selenomethionine (SM), 0.2 ppm sodium selenite (SS), 0.4 ppm SM, or 0.4 ppm SS, for a total of 5 dietary treatments in a factorial treatment arrangement. Selenomethionine (Sel-Plex) was provided by Alltech[5]. Sodium selenite was provided by International Nutrition[6]. Each treatment was assigned to 6 replicate cages. Birds were fed the dietary treatments for 6 weeks from 30-35 weeks of age. Hens were maintained on a 16:8 hr light:dark cycle throughout the trial. Each bird had approximately 600 sq. cm. of floor space (Alternative Design)[7]. Water was supplied ad libitum by nipple drinkers and the cage unit was located in a windowless, ventilated room.

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[4] Kumm's Kustom Pullets, NE, U.S.A.
[5] Alltech: 3031 Catnip Hill Pike, Nicholasville, KY 40356, U.S.A.
[6] International Nutrition: P. O. Box 27540, 7706 I Plaza, Omaha, NE, 68127, U.S.A.
[7] Alternative Design: 3055 Cheri Whitlock, P.O. Box 6330, Siloam Springs, AR, 72761-6330, U.S.A.

Diets

Diets (Table 1) were formulated to be isocaloric to provide 2949 kcal ME/kg feed and isonitrogenous to provide 17.5 % crude protein (CP). Birds were provided with *ad libitum* access to feed (80-100 g of feed per hen per day) and water throughout the study.

The five trace mineral premixes (Table 2) were added to the diets to meet the National Research Council (1994) trace mineral requirements for laying hens. Selenium from both sources was weighed, along with the other minerals, and 5 premixes were mixed separately and then added to 150 pounds of basal diet for each formulation and mixed again for 10-15 minutes. Samples were collected from each dietary mixing. A total of 3 mixings were done during the study. Dietary samples were stored at -20°C until chemical analysis was performed.

Measurements

Hen and Egg Parameters. Data collected included percent daily hen egg production and daily feed intake. Both egg production and feed intake were calculated on a hen/day basis. One days' total egg production was used to measure egg weight weekly and two eggs with similar weight were picked for measurement of Haugh units (Haugh, 1937), which was also done on a weekly basis. Egg weight and Haugh units were measured using Technical Services and Supplies (TSS) eggware[8]. Specific gravity was analyzed every week by placing all collected eggs from 1 days' egg production in graded NaCl solutions (1.070, 1.075, 1.080, 1.085, 1.090, 1.095 and 1.100). Hen weight was measured as a cage group at the start and the end of the trial, and average hen weight gain was calculated by cage.

[8] Technical Services and Supplies (TSS): York, England.

Egg Yolk and Albumen pH. Yolk and albumen pH were measured once every two weeks on fresh eggs and on eggs aged for two weeks in a cooler at 7° C. Two eggs per cage were collected for pH measurements (fresh and aged). The yolk was separated from albumen using an egg separator. Yolk and albumen were poured into different glass beakers and homogenized with a stirring bar, and then the sensing bulb of a glass pH electrode connected to a pH meter was used to measure the pH.

Egg Yolk Vitelline Membrane Strength. A texture analyzer machine (TA.XTPlus)[9] equipped with a 20-kg tension load cell and a crosshead speed of 10 mm/sec, was used to measure vitelline membrane strength of fresh eggs (Tharrington et al., 1998). A one millimeter width, rounded end, stainless steel probe was used to apply direct pressure to the membrane. Two eggs per cage were used for this measurement on a weekly basis. Eggs were broken, and yolks were separated from albumen and placed into a shallow dish. The height between the probe and base was set at 25-30 mm, and the calibration weight was set at 2 kg. Once the start button was pushed, the probe moved towards the yolk to penetrate through the yolk vitelline membrane in the equatorial region. Care was taken to avoid contact with the germinal disc or the chalazae. The force it took the probe to penetrate through the vitelline membrane strength was then measured directly in grams.

Yolk and Albumen Selenium Content. Three eggs per treatment were collected 3 times during the study for this measurement. Eggs were kept at a cooler at -7°C until they were

[9] Texture analyzer and exponent software from Texture Technologies Corp.: Ramona, CA, 25133 Hereford Dr. U.S.A.

sent to Alltech[5] for analysis. Eggs from the same treatments were pooled and yolk and albumen were separated before analysis was conducted.

Glutathione Peroxidase Determinations. Two hens per cage were euthanized at the end of the study, 1-2 g of liver and magnum cells were excised and immediately frozen in liquid nitrogen and stored at -80°C until further use. Enzymatic assay of glutathione peroxidase kit (EC. 1.11.1.9)[10] was used to measure the glutathione peroxidase (GPx) activity. At assay time, tissues were rinsed with a phosphate buffered saline and heparin solution to remove red blood cell contaminants. Tissues were then homogenized with a Tris-HCl, EDTA and Dithiothreitol homogenization buffer to yield a tissue homogenate concentration of 1 g tissue/5 ml homogenate. The homogenates were centrifuged at 8,000 g at 4°C for 15 minutes, and the supernatant was collected for each sample.

Glutathione peroxidase (GPx) acitivity was measured indirectly by a coupled reaction with glutathione reductase (GR). Oxidized glutathione (GSSG), produced upon reduction of hydrogen peroxide by glutathione peroxidase is recycled to its reduced state by glutathione reductase and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm. Under conditions in which the glutathione peroxidase activity is rate limiting, the rate of decrease in A_{340} is directly proportional to the glutathione peroxidase activity in the sample (Ursini et al., 1985). To measure GPx activity, 10 µl of diluted tissue homogenate were added in triplicate to 96 well plates and 235 µl of a reaction cocktail containing ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced, (ß-NADPH), Glutathione Reductase Enzyme and

[10] Sigma Aldrich: Phone: 800-325-3010. Fax: 800-325-5052.

Glutathione were added to each well. Negative control samples and positive control samples (containing known concentrations of GPx) were also added to each plate. The plate was shaken for 5 seconds, and then sample absorbance at 340 nm (A_{340nm}) was measured on a microplate reader[11]. Next, 5 µl of a hydrogen peroxide solution were added to each well and the A_{340nm} was recorded every minute for 5 minutes.

To calculate the final results, the change in absorbance (ΔA_{340}) per min for each sample was calculated using the most linear portion of the curve (time=0 minutes to time= 1minute). Glutathione peroxidase activity (units GPx activity/ min*µg tissue) was calculated using the equations

GPx Activity/ min *ml sample homogenate = [(ΔA_{340})/min Sample-(ΔA_{340})/min Blank x 2^A x total assay volume x dilution factor]/ [6.22^B x sample volume]

[A]µmoles of GSH produced per µmole of ß-NADPH oxidized

[B]Millimolar extinction coefficient of ß-NADPH at 340 nm

GPx activity/ min * µg tissue = [GPx Activity/ min *ml sample homogenate]/[µg tissue/ ml homogenate]

Chemical Analysis. Dietary samples were collected from the feed mixing at the start of the trial and analyzed for Ca (927.02), P (965.17), and crude protein (Kjeldahl Method) (988.05) (A.O.A.C., 1984). Selenium analysis of the diets, yolk and albumen were analyzed by Alltech[5] using a PSA Millenium Excalibur system[12], which utilizes continuous flow vapour/hydride generation atomic fluorescence to analyze for selenium (Wallschlager et. al, 2001).

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[11] Microplate Reader: BioTek Instruments Inc. Winooski, VT.

[12] P S Analytical: Arthur House, Crayfields Industrial Estate, Main Rd, Orpington, Kent, BR5 3HP, UK.

Statistical Analysis. All data were analyzed as a repeated measure using GLIMMIX procedure of SAS (SAS 9.2, 2008). The experimental design was repeated measures, randomized complete block design. Blocking was implemented in order to reduce the effect of temperature stratification in the cage unit. Blocks were considered a random effect, dietary treatments were considered fixed. A total of five dietary treatments were assigned (0, 0.2 ppm selenomethionine (SM), 0.2 ppm Sodium Selenite (SS), 0.4 ppm SM, or 0.4 ppm SS) in a factorial treatment design. Cages were the experimental units, with 4 hens per cage, and 6 cages per treatment. Average values for the variables were generated and subsequently analyzed separately to determine differences between combinations of treatments. The following model was used to determine differences between treatments groups

$$Y_{ijklm} = \mu + R_{k+} \alpha_i + \beta_j + \alpha\beta_{ij} + \tau_l + \alpha\tau_{il} + \beta\tau_{jl} + \alpha\beta\tau_{ijl} + \varepsilon_{ijklm}$$

Where

Y_{ijklm} = Variable measured.

μ = Overall mean.

R_l = Effect of l^{th} block.

α_i = Selenium level effect.

β_j = Selenium source effect.

$\alpha\beta_{ij}$ = Interaction effect of Se level and source.

τ_l = Time effect.

$\alpha\tau_{il}$ = Interaction effect of Se level and time.

$\beta\tau_{jl}$ = Interaction effect of Se source and time.

$\alpha\beta\tau_{ijl}$ = Intreraction effect of Se source and level and time.

ε_{ijklm} = Residual error.

The separation of means was done using LS means statement with the pdiff option.

The data for Glutathione peroxidase activity in the liver and magnum were analyzed using the model

$Y_{ijkl} = \mu + R_{k+} \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijkl}$

Where

Y_{ijkl} = Variable measured.

μ = Overall mean.

R_k = Effect of k^{th} block.

α_i = Selenium level effect.

β_j = Selenium source effect.

$\alpha\beta_{ij}$ = Interaction effect of Se level and source.

ε_{ijkl} = Residual error.

The separation of means was done using LS means statement with the pdiff option.

RESULTS

Table 1 shows the diet composition and nutrient composition of the semi-purified corn starch-soybean meal basal diet and the analyzed protein, Ca, and P. The diet contained 46.94% corn starch, and 37.36% soybean meal. Total Se analyzed was 0.42 ppm whereas the calculated Se content was 0.09 ppm. Table 2 shows the Se source treatment combinations and analyzed Se content. Calculated levels of Se in the diet were 0.0 ppm, 0.2 ppm selenomethionine (SM), 0.2 ppm sodium selenite (SS), 0.4 ppm SM, and 0.4 ppm SS, whereas actual results were 417.5, 483.83, 601.17, 822.83, or 591.33

ppm Se, respectively. The basal diet was much higher than expected likely due to high soybean meal Se content.

When looking at the main effects of Se source and Se level on feed intake, feed intake was significantly affected by Se source ($P = 0.0397$) (Figure 1), with higher feed consumption when supplementing hens with selenomethionine (SM) compared to sodium selenite (SS) (77.22 vs. 72.20 g/hen/d, respectively). Higher levels of Se increased feed intake (Figure 2); feed intake increased from 70.85 g/hen/d for the diet supplemented with 0.2 ppm Se to 78.58 g/hen/d for the diet supplemented with 0.4 ppm Se ($P = 0.0034$). Dietary treatments had a significant effect on feed intake (Table 3) ($P = 0.0054$); hens supplemented with Treatment 4 consumed more feed (80.60 g/hen/d) compared to the other combination of treatments although this intake was not significantly different from Treatments 1 or 2.

Egg production followed the same trend as feed intake (Table 3). There was a significant Se level effect on egg production, with significantly higher egg production (83.22%) when using 0.4 ppm Se (71.46%) compared to when using 0.2 ppm Se ($P = 0.0189$) (Figure 3). There was a significant treatment effect on egg production with the highest egg production attained when supplementing hens with 0.4 ppm SM (87.04%), but this was not significantly different from Treatments 1 or 5 ($P = 0.0502$).

Main effects of treatments on hen weight gain were not significant ($P =0.2557$) (Table 3), but there was a control vs. Treatments 2, 3, 4, and 5, with the control having more weight loss than the other treatments (-205.8 vs. -143.6, -101.4, -106.7, -106.1, respectively) ($P = 0.0380$).

Egg weight ($P = 0.5313$), Haugh units ($P = 0.7857$), specific gravity ($P = 0.4370$), vitelline membrane strength ($P = 6575$), and fresh ($P = 0.2495$; $P = 0.3875$) and aged ($P = 0.9126$; $P = 0.7592$) yolk and albumen pH, respectively, were not affected with dietary treatments (Table 4).

Table 5 shows the results of treatment effect on Se content of yolk and albumen of eggs. There was a treatment effect on yolk Se when comparing the control diet vs. Treatments 2, 3, 4, and 5 (1.24 vs. 1.57, 1.41. 1.63, 1.49 µg/g, respectively) ($P = 0.0497$) (Figure 4), as Se level increased in the diet, yolk Se content increased significantly but SM always gave higher Se content compared to SS, and both Se sources gave higher amounts of Se in yolk than the basal level. There was an interaction effect between Se source and level affecting albumen Se content in eggs ($P = 0.0358$) (Figure 5). Albumen Se content increased from 2.33 to 2.93 µg/g when SM levels increased in the diet but not with increasing SS (1.90 to 1.95 µg/g). There was a significant treatment effect on Se content of albumen ($P = 0.0054$); the highest level of Se in albumen was attained when hens were fed SM at 0.4 ppm, which had the highest Se content when analyzed (822.83 ppb) (Figure 6).

Neither selenium level nor source had any effect on GSH-Px activity in the liver ($P = 0.6853$) or magnum ($P = 0.4748$) of hens in this study (Table 6).

DISCUSSION

The hypothesis for this study was based on the hypothesis that supplementation of Se to a basal diet low in Se (semi-purified diet) would bring more significant results in egg production and quality parameters in laying hens than when supplementation is

applied to a corn-soybean meal basal diet that already has adequate Se. Unfortunately, diet analysis was done at the end of the trial, which could have revealed the unexpected high Se level from the beginning if analysis was conducted from the start of the trial. Using the feed formulation program[13] to formulate the diet for this trial, Se content was predicted to be 0.09 ppm (Table 1), which is lower than the normal corn-soybean meal basal diet in Nebraska and the Dakotas that are known for the high Se level in their soil and the high Se level of corn and soybean grown there as a result. But actual results of Se content were much higher than anticipated. Treatment 4 supplemented with 0.4 ppm selenomethionine (SM) gave the highest Se content when analyzed of 822.83 ppb, followed by treatment 3 which was supplemented with 0.2 ppm sodium selenite (SS) (601.17 ppb). Treatment 5 gave 591.33 ppb Se when analyzed, and Treatments 1 and 2 gave 417.5 and 483.83 ppb, respectively. This was likely due to the higher levels of soybean meal used, originated from NE, with levels of 0.54 ppm Se compared to 0.1 ppm from other places (Leeson and Summers, 2001). Soybean meal percentage in the diet was nearby 10-15% higher than its normal percentage in a corn-soybean meal basal diet in a laying hen ration (37.36 vs. around 20-25%). So rather than having a low basal Se level, the trial ended up testing very high levels of Se, well above NRC (1994) and FDA regulations (1997), so that, ironically, what started as a requirement study ended up being more of a toxicity study.

Previously, Bunk and Combs (1981) conducted experiments to determine the effects of oral Se administration on Se-deficient chicks, and they found that

[13] ECO-MIX: Logic soft solution, 101, H No. 1-1-565 Raghavendra Apts, New Bakaram, Musheerabad, Hyderabad-20 Adnhra Pradesh, India. www.ecomixonline.com/

administration of SM significantly increased feed consumption compared to SS, which is in agreement with our findings. These results also agree with the findings of Zuberbuehler et al. (2002), who concluded from their study that young Se-deficient laying hens reduce their Se deficit if they have a choice between a Low-Se and a High-Se diet by preferentially selecting the High-Se diet. The low feed intake noticed for all treatments in this trial compared to normal feed intake for hens reared under normal conditions (105 g/hen/d) according to the commercial management guide for Bovans White laying hens is due to the use of semi-purified diet. Physical structure of the feed (particle size, feed form, and inclusion of whole grains) influences GIT (gastro-intestinal tract) structure, composition of the microflora, nutrient digestibility, and feed intake (Mateos et al., 2006). Finely ground cereals or starch based diets might be detrimental for mucosal cell growth and motility of the GIT because they might produce atrophy of the gizzard, a major regulator of intestinal motility (Nir et al., 1994; Jones and Taylor, 2001). Kilburn and Edwards (2004) have found that coarse soybean meal increases bone ash of broiler chicks, probably through an improvement in mineral utilization, and also improves growth and feed efficiency when used in semi-purified diets. Egg production followed the same trend as feed intake, when feed intake was higher egg production was higher as well.

The lack of treatment effect on egg quality parameters may be a result of the unexpected high level of Se in the basal diet that met Se requirements, and that further supplementation had no additional effect on those variables.

The results of egg Se content agree with the results of studies conducted by Surai (2000), which showed that egg selenium content can be easily increased when SM is

included in the diet at a level to provide 0.4 ppm Se, and that Se content increased from 7 to 43 µg in the egg by adding up to 0.8 ppm organic Se to the basal diet.

The results of this study indicate that higher selenium levels of 0.8 ppm as analyzed did not adversely affect any of the production parameters. Combs (2000) reported that using Se in the form of high-quality Se-enriched yeast in the chicken diet at 1.2 ppm results in Se content in the egg of up to 200 µg. Interestingly, in our study the highest level of Se attained in analysis increased feed intake significantly and gave higher Se content of albumen that reached 2.2 µg/g. The yolk Se content averaged 1.47 µg/g for all treatments. So an egg that has 50 grams of total albumen and yolk from this study could supply around 100 µg Se, a novel finding that was not expected. According to Whanger (2004), the maximum safe dietary selenium intake for humans is 819 µg/day which is 8 times higher than the level reached in this study per egg. The RDA for Se in the U.S. is 55 µg/day for adult males and females (Fisinin, 2007). So one egg from our study contains 2 times the daily Se requirement.

In conclusion, higher levels of Se that reached 0.8 ppm (as analyzed) did not have negative effects on egg quality, rather, it increased egg yolk Se content to a level that can contribute 2 times the human RDA for this nutrient by consuming 1 egg a day. It is recommended that FDA should increase the level of Se inclusion in the diet of laying hens to assist the value of high Se ‘designer eggs’ in the market place.

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Table 4.1 Diet composition and nutrient content of the standard corn starch-soybean meal diet.

Ingredients	**% Diet**	
Corn Starch	46.94	
Soybean Meal- 47%	37.36	
Tallow	1.81	
Shell and Bone	4.49	
Dicalcium Phosphate	1.91	
Salt, White	0.42	
DL- Methionine	0.34	
Lysine	0.11	
Vitamin Premix[1]	0.20	
Mineral Premix[2]	0.10	
Composition	**Calculated Nutrient Composition**	**Analyzed Nutrient Composition**
ME, kcal/kg	2949	-
Protein, %	17.5	23.36
Methionine, %	0.55	-
Met+Cys	0.82	-
Lysine, %	1.15	-
Ca, %	4.1	4.2
Total P, %	0.73	0.62
Se (ppm)	0.09	0.42
Sodium	0.17	

[1] Provided per kilogram of diet: Vitamin A 29,964,000 IU; Vitamin D 35,200,000 IU; Vitamin K 35,200 mg; Vitamin B-12 1,320 mg; Riboflavin 798,336 mg; Niacin 498,960 mg; Pantothenic acid 323,855.4 mg; Folic acid 99,792 mg; Vitamin B-6 821,286.4 mg; Vitamin E 88,000 IU; Choline 598,400 mg; Thiamin 88,000 mg; Biotin 2,200 mg.

[2] Provided per kilogram of diet: Copper, 8.75 mg from copper sulfate; zinc, 35 mg from zinc sulfate; iodine, 0.035 mg from organic iodine; manganese, 20 mg from manganese sulfate; iron, 45 mg from iron sulfate.

Table 4.2 Dietary Se source treatment combinations and analyzed Se content.

Treatment	Source		Analyzed Se content
	Selenite (ppm)	Selenomethionine (ppm)	(ppb)
1 (Control)	0.0	0.0	417.5
2	0.0	0.2	483.83
3	0.2	0.0	601.17
4	0.0	0.4	822.83
5	0.4	0.0	591.33

[3] Diet Se was analyzed at Alltech Labs using a PSA Millenium Excalibur system, which uses continuous flow vapour/hydride generation atomic fluorescene to analyze for selenium (Wallschlager et al., 2001), Dec/2010.

Table 4.3 Treatment effect on feed intake, egg production, and hen weight gain.

Treatment	Source		Feed Intak	Egg Production	Hen Wt.
	Selenite (ppm)	Selenomethionine (ppm)	(g/hen/d)	(%)	(g)
1 (Control)	0.0	0.0	78.24[ab]	82.60[ac]	-205.8
2	0.0	0.2	73.85[cb]	72.06[a]	-143.6
3	0.2	0.0	67.84[c]	70.86[a]	-101.4
4	0.0	0.4	80.60[a]	87.04[bc]	-106.7
5	0.4	0.0	76.57[ab]	79.40[ac]	-106.1
***P* value**			0.0054	0.0502	0.2557
SEM[4]			2.1708	4.1574	38.11
Main Effects					
Se Source					
Selenomethionine			77.22[a]	79.55	-125.1
Selenite			72.20[b]	75.13	-103.8
SEM			2.2289	4.4711	36.88
Se Level (ppm)					
0.2			70.85[a]	71.46[a]	-122.5
0.4			78.58[b]	83.22[b]	-106.4
SEM			2.2289	4.4711	36.88
***P* values**					
Se Source			0.0397	0.3380	0.5706
Se Level			0.0034	0.0189	0.6684
Se Source x Se Level			0.6662	0.4827	0.5805
Control vs. Treatments 2, 3, 4, 5			0.1614	0.2680	0.0380

[a,b,c] Different superscripts within one column are significantly different at $P \leq 0.05$.
[4] SEM: Standard Error of Mean.

Table 4.4 Treatment effect on egg quality parameters.

Treatment	Source		Egg Wt.	HU[5]	Specific Gravity	VMS[6]	Yolk pH		Albumen pH	
	Selenite (ppm)	Selenomethionine (ppm)	(g)			(g)	Fresh	Aged	Fresh	Aged
1	0.0	0.0	56.47	91.21	1.086	6.76	6.038	6.311	8.338	8.956
2	0.0	0.2	54.31	91.24	1.088	6.60	6.052	6.293	7.982	8.975
3	0.2	0.0	54.40	91.22	1.084	6.86	6.118	6.329	8.300	8.959
4	0.0	0.4	53.99	88.92	1.086	6.75	6.100	6.296	8.449	8.995
5	0.4	0.0	53.85	93.16	1.085	6.71	6.096	6.269	8.401	8.962
***P* value**			0.5313	0.7857	0.4370	0.6575	0.2495	0.9126	0.3875	0.7592
SEM			1.2177	2.3347	0.0015	0.1130	0.0282	0.0427	0.1832	0.0242
Main Effects										
Se Source										
Selenomethionine			54.64	90.61	1.087	6.68	6.076	6.296	8.215	8.985
Selenite			53.72	92.47	1.085	6.79	6.106	6.299	8.350	8.968
SEM			1.0153	2.0792	0.0014	0.1072	0.0251	0.0421	0.1957	0.0216
Se Level (ppm)										
0.2			54.82	91.87	1.086	6.73	6.084	6.312	8.140	8.968
0.4			53.54	91.21	1.085	6.73	6.098	6.283	8.425	8.985
SEM			1.0153	2.0792	0.0014	0.1072	0.0251	0.0421	0.1957	0.0217
***P* values**										
Se Source			0.3799	0.3833	0.1472	0.3275	0.2487	0.9456	0.5030	0.4505
Se Level			0.2227	0.7540	0.6415	0.9739	0.5780	0.4916	0.1707	0.4492
Se Source x Se Level			0.9884	0.2048	0.4616	0.1899	0.1953	0.5023	0.3656	0.8899
Control vs. Treatments 2, 3, 4, 5			0.0913	0.9778	0.9069	0.8219	0.0975	0.7519	0.7802	0.5326

[5] HU: haugh units.
[6] VMS: vitelline membrane strength.

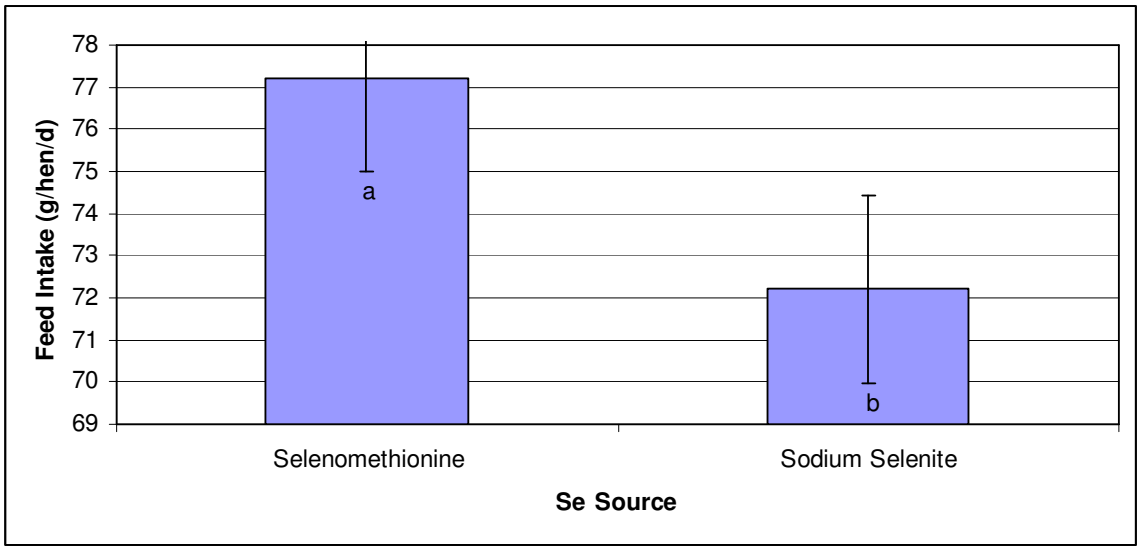
Table 4.5 Treatment effect on Se content of yolk and albumen of egg.

Treatments	Source		Se Content	
	Selenite (ppm)	**Selenomethionine** (ppm)	**Yolk**	**Albumen** (µg/g)
1	0.0	0.0	1.24	1.98[a]
2	0.0	0.2	1.57	2.33[a]
3	0.2	0.0	1.41	1.90[a]
4	0.0	0.4	1.63	2.93[b]
5	0.4	0.0	1.49	1.95[a]
***P* Value**			0.1857	0.0054
SEM			0.0985	0.1122
Main Effects				
Se Source				
Selenomethionine			1.52	2.54[a]
Selenite			1.45	1.92[b]
SEM			0.0860	0.0960
Se Level (ppm)				
0.2			1.41	2.03[a]
0.4			1.56	2.44[b]
SEM			0.0860	0.0960
***P* values**				
Se Source			0.6038	0.0039
Se Level			0.2537	0.0230
Se Source x Se Level			0.5788	0.0358
Control vs. Treatments 2, 3, 4, 5			0.0497	0.0604

Table 4.6 Treatment effect on liver and magnum GSH-Px activity.

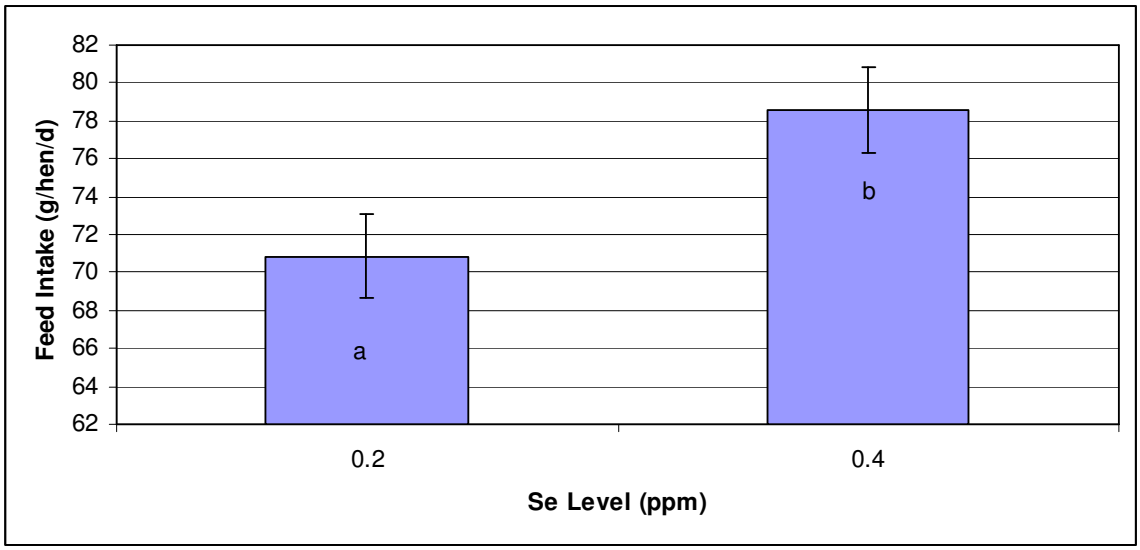
Treatments	Source		GSH-Px Activity	
	Selenite (ppm)	**Selenomethionine** (ppm)	**Liver** (nmol/min/ml)	**Magnum**
1	0.0	0.0	164.3	73.34
2	0.0	0.2	165.6	112.5
3	0.2	0.0	188.1	82.79
4	0.0	0.4	162.8	98.51
5	0.4	0.0	160.5	79.60
***P* Value**			0.6853	0.4748
SEM			14.9	17.88
Main Effects				
Se Source				
Selenomethionine			164.2	105.5
Selenite			174.3	81.20
SEM			13.09	18.01
Se Level (ppm)				
0.2			176.9	97.65
0.4			161.6	89.05
SEM			13.09	18.01
***P* values**				
Se Source			0.4492	0.1921
Se Level			0.2583	0.6382
Se Source x Se Level			0.3529	0.7670
Control vs. Treatments 2, 3, 4, 5			0.7704	0.2946

Figure 4.1 Main effect of Se source on feed intake.



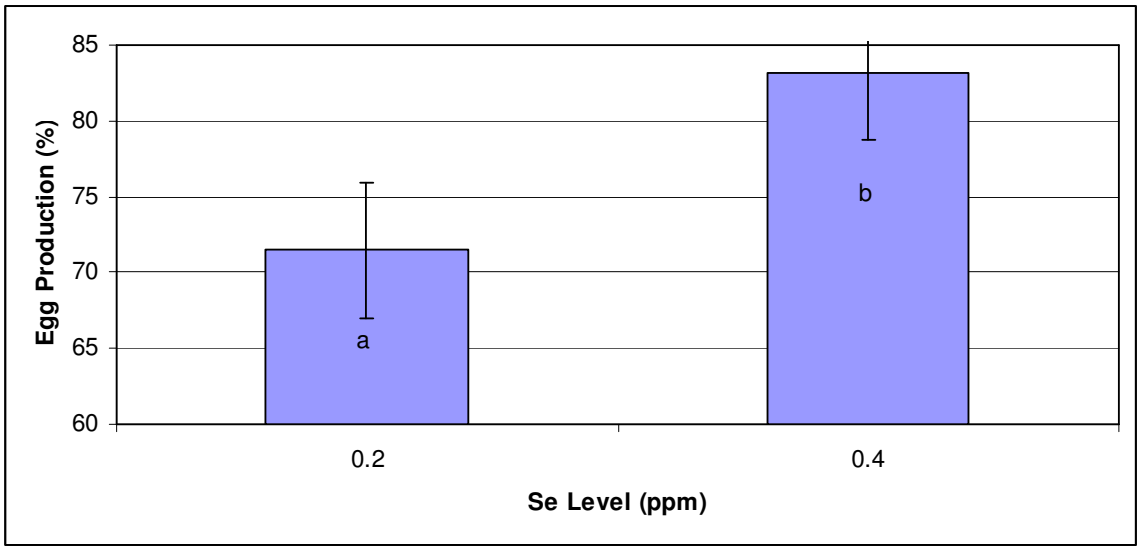
P = 0.0397

Figure 4.2 Main effect of Se level on feed intake.



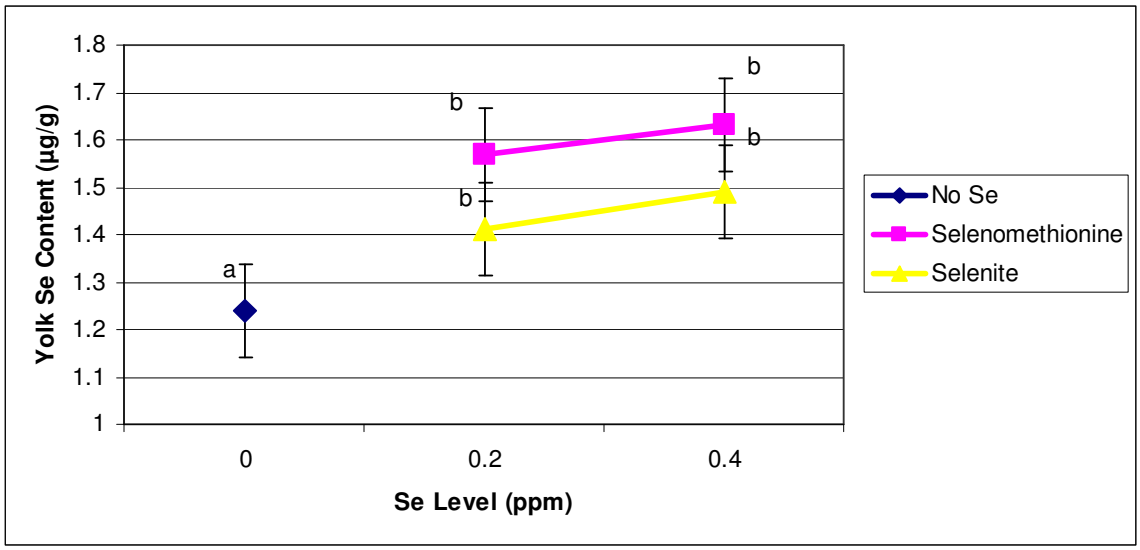
P = 0.0034

Figure 4.3 Main effect of Se level on egg production.



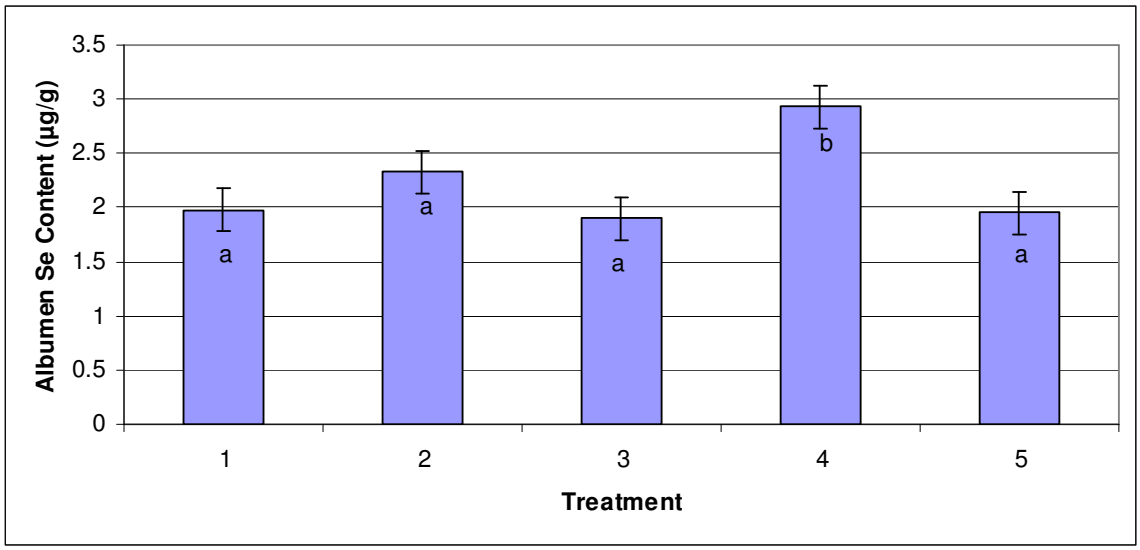
P = 0.0189

Figure 4.4 Effect of control vs. treatments 2, 3, 4, and 5 on yolk Se content.



P = 0.0497

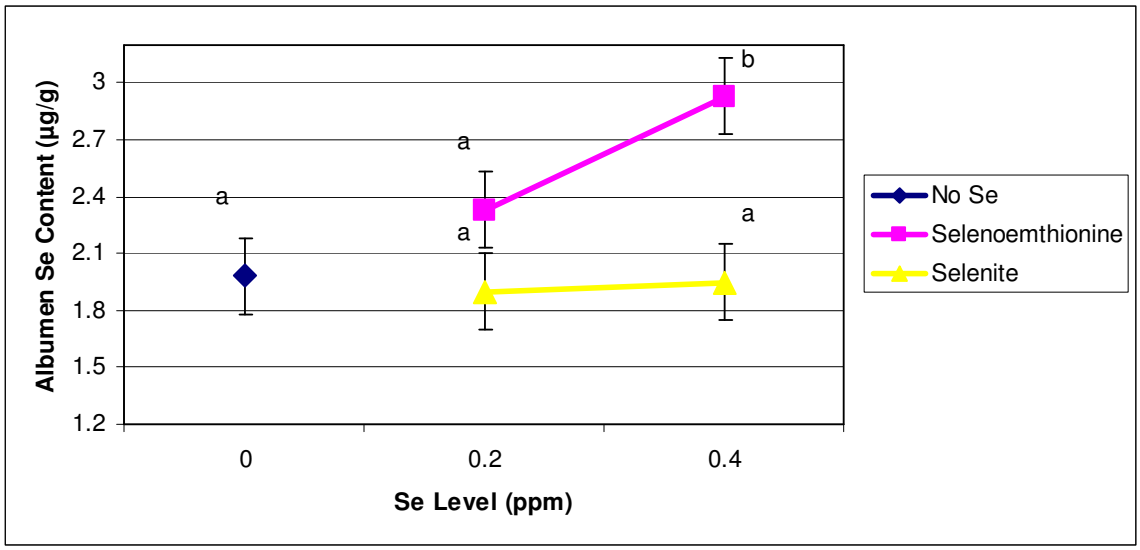
Figure 4.5 Treatment effect on albumen Se content.



P = 0.0054

Treatment 1: No Se, treatment 2: 0.2 ppm selenomethionine, treatment 3: 0.2 ppm sodium selenite, treatment 4: 0.4 ppm selenomethionine, treatment 5: 0.4 ppm sodium selenite.

Figure 4.6 Interaction effect of Se source and level on albumen Se content.



P = 0.0358

SUMMARY AND CONCLUSION

The main point of this research was to improve egg quality parameters and egg yolk vitamin E and Se content. The three studies conducted in this dissertation addressed the impact of supplementing high levels of vitamin E, inorganic Se (sodium selenite- SS) and organic Se (selenomethionine- SM) on egg production and quality variables, antioxidant status in the liver, shell gland and magnum of the hen as indicated by glutathione peroxidase activity (GSH-Px), and optimum yolk vitamin E and Se content. The hypothesis behind the research was that increasing vitamin E and/or Se in the diet of the laying hen will increase their deposition in the egg yolk, yolk vitelline membrane, and albumen (with increasing Se alone), even if the basal diet has adequate amounts of these nutrients, and this will decrease oxidation potential in the egg and further improve quality. Also, Se inclusion as part of the enzyme GSH-Px may increase the activity of this enzyme in different tissues, thereby reducing oxidative stress in the hen. We chose to study the activity of GSH-Px particularly in the liver, magnum, and shell gland because of their importance in yolk, albumen and egg shell synthesis that may contribute to egg quality. A comparison of organic and inorganic sources of Se was also conducted, as some have shown that organic Se is more bioavailable and safer to use. High levels of vitamin E and Se greater than NRC requirements were tested in order to investigate the effect of the treatment combinations on egg quality. Do we really need higher amounts of vitamin E and Se than stated in the NRC? Do we get higher production and/or egg quality parameters when supplementation is done at the levels chosen? And is this economical? Those questions were addressed in each of the three studies conducted for this dissertation.

The results of our studies demonstrate that supplementing high levels of vitamin E and Se in the hen diets did not improve all egg quality parameters; the treatments had a positive effect on α-tocopherol and Se deposition in the yolk in the first trial. The first trial also showed a decrease in the effect of storage on yolk quality indicated by aged yolk pH with vitamin E. In the second trial, there was an enhancement in feed intake and egg production when using higher levels of SM but it was always not significantly different from the 0.2 ppm SS supplementation. There was a tendency to improve egg albumen quality with aging indicated by aged albumen pH with higher Se levels. Using 0.4 ppm SM increased the strength of the vitelline membrane although this was not significantly different from the 0.2 ppm SS suppelementation. The last trial showed that very high Se levels in a semi-purified diet increased feed intake and egg production. The Se content of yolk and albumen increased as a result of Se supplementation at 0.8 ppm SM (as analyzed).

The main finding of the research conducted is that supplementing high levels of vitamin E and Se increased their deposition in the egg to a point where their combination at the highest levels used (100 IU/kg vitamin E with 0.5 ppm SS) would provide near 30% of the RDA for human consumption (5 IU vitamin E/egg, and 22 µg Se/egg), and dietary levels as high as 0.8 ppm Se from SM (as analyzed in the last trial), it was still safe and could provide consumers with their RDA of Se and give profit to the producer.

In order to produce the 'designer eggs' enriched with Se at levels that significantly contribute to the RDA for human consumption, FDA should consider Se inclusion in the laying hen diet to a level higher than 0.3 ppm, esp. that no negative effects were shown from using the highest levels in this research.

FUTURE RESEARCH

As more commercial eggs are further processed, the vitelline membrane strength is important to prevent contamination of the albumen by yolk which can cause huge economical loss for the egg-breaking industry. So future research should be directed towards studying the composition of yolk vitelline membrane, measuring Se content of the membrane, and relating that to the strength of the membrane and interior quality of fresh vs. aged eggs.

While many essential nutrients can be obtained in tablet or capsular forms, it is generally held that their supply in normal dietary components is more valuable. In this respect, more emphasis should be given to supplementation of Se and vitamin E at high levels to produce eggs enriched with these nutrients for human consumption. Eggs can work as a food vehicle to supply a good percentage of the human RDA of these nutrients. In general, future research should focus on enhancing the quality of eggs, fresh and aged, for both, the producer, to get a net profit while producing high quality eggs for the consumer.

APPENDICES

Appendix A. Gross Energy Determination of feed

1- Approximately 1 g of the sample of feed was pelleted in Parr pellet press.

2- The combustion capsule was weighed accurately, and with a forceps, the pellet was placed in the capsule and was weighed.

3- In case of fecal material, 0.2g of feces was weighted in the capsule with addition of 0.4g of mineral oil.

4- The bomb calorimeter was charged with the sample with an approximately 10 cm long fuse (nickel alloy wire) was attached to the electrodes and the wire loop was allowed to touch the surface of the charge containing the capsule.

5- Then, 1ml of distilled water was added to the bottom of the bomb which functions as a sequestering agent and absorbent.

6- Then, the bomb was loaded and was charged with oxygen with pressure of 30 atmospheric pressure.

7- The calorimetric bucket was filled with 2 liters of distilled water. Then the bucket was set in the calorimeter and the lifting handles were attached to the two holes in the side of the screw cap and the bomb was lowered into the water with its feet spanning the circular boss in the bottom of the bucket.

8- The bomb clorimeter cover was closed and the thermometer bracket was lowered.

9- The power switch was turned on and the calorimeter was run for 5 min while the controller brings the jacket temperature to equilibrium with the bucket.

10- After the temperature was equalized, the initial temperature was recorded. Fire the bomb and the final temperature was recorded once the temperature reaches a stable maximum and remains constant for at least two minutes.

11- Bomb then removed and the interior of the bomb was washed with distilled water.

12- The unburned piece of fuse wire was measured.

13- The bomb washings were then titrated with standardized sodium carbonate solution using methyl red indicator till the solution changes from red to yellow.

14- The volume and the normality of sodium carbonate solution used were recorded.

15- The gross energy of combustion was calculated from the equation:

$$GE = tW\text{-}e_1\text{-}e_2/m;$$

t is the temperature difference (final-initial).

W is the energy equivalent of calorimeter.

e_1 is the correction in calories for heat of formation of nitric acid.

e_2 is the correction in calories for heat of combustion of fuse wire.

m is the mass of the sample in grams.

e_1 = Normality of actual Na_2CO_3/0.0725 x Nomality of ideal Na_2CO_3 X C_1

e_2= 2.3 x C_2.

C_1 = ml of standard Na_2CO_3 used in acid titration.

C_2 = net length of fuse wire burned, cm.

Appendix B. Nitrogen Determination of feed

The amino nitrogen is oxidized by sulfuric acid in the presence of a catalyst to ammonium sulfate. Sodium hydroxide is added, converting the ammonium ion to NH_3, which is collected by distillation. The NH_3 is then quantitatively titrated.

Reagents:

1. 50 % Sodium Hydroxide.
2. 0.1% Bromocresol Green.
3. 0.1% Methyl Red.
4. 4% Boric acid.
5. 0.15 N HCl for titration.

Digestion: 1.0 g of feed sample or 0.5g of feces sample was weighted into Kjeltec digestion tube along with two blank tubes and two kjeltabs (catalyst) were added to the tubes. 15 ml of concentrated H_2SO_4 was added and swirled gently to wet the sample. The samples were digested in a block digestor set at 420°C for 60 min. The digestor was then allowed to cool for 5 min and 80 ml of water was added, shaking it to dissolve any crystals that may form.

Distillation: The digestion tube was placed in the distillation apparatus and receiver flasks were filled with 25 ml of red boric acid solution. The distillation apparatus adds alkali and distill the ammonia over into the receiver flask. After distillation the solution in the receiver flask turned green.

Titration: The solution in the receiver flask was titrated against 0.15 N HCl until a purple-rose endpoint is reached and the volume of HCl added was recorded.

The percentage protein in the sample was calculated by:

% protein = (ml HCl – ml blank) x normality of HCl x 14.007 x 6.25/g of sample x 10;

where 6.25 is the constant for calculating % crude protein from % nitrogen.

Appendix C. Calcium Determination of feed

4 g of dried feed samples in a crucible was placed in muffle furnace. Ashed for 6h at 600°C. The samples were then cooled and 10 ml of 3 M HCl was added to the ash in the crucible. The samples were boiled for 10 min, cooled and washed into a 100 ml volumetric flask. Samples were filtered into a plastic tube and were used for calcium assay.

Reagents:

1. 0.3 N HCl.
2. 15 g strontium chloride in 100 ml water. This will contain 50 mg Sr/ml.
3. Calcium standard solutions.
4. Blank (water).
5. Diluted samples (1:50000).

The standards were prepared by pipetting 0.1, 0.2, 0.3, 0.4 and 0.5 ml of stock Ca (1000 ppm) into five different 100 ml volumetric flasks. To each of these flasks add 10 ml water, 4 ml 50 mg/ml strontium solution and made up the volume with 0.3 N HCl. Blank was prepapred by pipetting 10 ml water and 4 ml 50 mg/ml strontium solution and made up the volume with 0.3 N HCl.

The samples and standards were allowed to stand for 1 hr and were analyzed using Atomic Absorption Spectrophotometer under set conditions.

% Ca = Absorbance x dilution factor/10^6 x sample wt. X 100.

Appendix D. Phosphorus Determination of feed

4g of dried feed samples in a crucible was placed in muffle furnace. Ash for 6h at 600°C. The samples were then cooled and 10 ml of 3 M HCl was added to the ash in the crucible. The samples were boiled for 10 min, cooled and washed into a 100 ml volumetric flask. Samples were filtered into a plastic tube and were used for phosphorus assay.

Reagents:

1. 2 L of molybdovanadate.
2. Phosphorus standard (2mg P/ml). Dissolve 8.788 g potassium. orthophosphate, dihydrogen (KH_2PO_4) in water and dilute to 1 liter. Working standards contain 10, 20, 30, 40 and 50 µg P/ml.
3. Diluted sample solution (1:1000).
4. Blank (4 ml molybdovanadate to 1ml water).

4ml of molybdovanadate reagent was added to 1ml of each standard and sample and mixed well. Allowed to stand for 10 min and the absorbance was read at 400 nm on UV-VIS Shimadzu spectrophotometer.

% P = Absorbance x dilution factor/10^6 x sample wt. X 100.