

Abstract

Upton, Jr., J. Robert. The effects of selenium supplementation on performance and antioxidant enzyme activity in broiler chickens. (Under the direction of Drs. F. W. Edens and P. R. Ferket)

In August 2000, organic selenium in a selenized yeast, (Sel-Plex™, Alltech Biotechnology Center, Nicholasville, KY) that contained high levels of selenomethionine was approved by the United States Food and Drug Administration as a source of selenium (Se) supplementation for broiler chickens. The previously reported positive responses to the presence of selenomethionine in Se yeast supplemented feed have increased the interest in use of organic Se in all phases of poultry production. Thus, a series of experiments were conducted to compare the influences of organic and inorganic sources of Se (Se yeast or sodium selenite) on production performance parameters. Body weights, feed conversion ratio (FCR), cut up parts yield as a percentage of carcass weight, drip loss from breast meat, and serum thyroid hormones were measured up to 6 weeks of age.

Body weight at 42 days was increased in Se yeast fed broilers as compared to those in the no supplemental Se or sodium selenite treatment groups. The combination of sodium selenite and selenized yeast was no more effective than selenized yeast alone on body weight. FCR were improved by all Se sources, with the selenized yeast and selenized yeast + sodium selenite treatments being superior to sodium selenite only fed birds.

On a percentage of carcass weight, yields of viscera, feet and neck were higher in selenized yeast treated birds. Yields of leg and *pectoralis major*, as percentages of carcass weight, were increased and decreased, respectively, in the selenized yeast treated birds. There was an increase in breast meat drip loss when birds were fed sodium selenite as compared to those fed the selenized yeast or no supplemental Se treatments, suggesting that

prooxidant properties of sodium selenite supplementation may be associated with increased moisture loss from processed breast meat.

The serum T_4 levels were higher in birds within the no supplemental Se treatment as compared to those supplemented with sodium selenite or selenized yeast. The ratios between serum T_4 and T_3 indicate that selenized yeast treatment facilitated the extra-thyroidal conversion of T_4 to T_3 .

The Se status of broilers influenced by dietary Se sources may increase the bird's ability to overcome the adverse effects of reactive oxygen metabolites (ROM). The addition of peroxidized fat to poultry diets produces a state of oxidative stress and can increase the level of ROM that must be reduced in the bird. A second study was conducted to evaluate the positive responses to selenized yeast as influenced by an improved status of the antioxidant enzymes, glutathione peroxidase (GSH-Px) and glutathione reductase (GR). This study evaluated the effects of feeding graded levels of oxidized poultry fat on blood and hepatic GSH-Px and hepatic GR activity in broiler chickens given inorganic or organic forms of dietary Se.

Neither fat oxidation nor Se source significantly altered the BW and FCR of broilers, or their activity of hepatic GR. Blood GSH-Px was influenced significantly by both fat and Se source, but the fat X Se source interaction was not significant. There was a Se source effect on the hepatic GSH-Px activity with sodium selenite causing an elevated GSH-Px activity, even in the basal diets with no added oxidized fat. There was no peroxide level effect on GSH-Px in the sodium selenite group, but GSH-Px activity in the Se yeast group did not increase until dietary peroxide level was at the highest inclusion rate.

Because elevated GSH-Px is indicative of oxidative stress, the dietary Se yeast supplementation resulted in better Se status in broilers than in birds fed sodium selenite. Only dietary inclusion of the highest level of oxidized fat was sufficient to impose oxidative stress in the Se-yeast fed birds to induce hepatic GSH-Px activity. These results suggest that the dietary Se supplied in an organic form (selenomethionine) may improve Se status in broilers, leading to greater resistance to oxidative stress than when an inorganic form of Se (sodium selenite) is fed.

The results from these studies suggest that selenomethionine from selenized yeast may be a superior form of Se for poultry. The beneficial effects of selenomethionine on production performance and the antioxidant enzyme profile of broiler chickens appear to support this conclusion.

The Effects of Selenium Supplementation on Performance and Antioxidant Enzyme Activity in Broiler Chickens.

By

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A thesis submitted to the Graduate Faculty of
North Carolina State University in partial
fulfillment of the requirements for the
Degree of Master of Science

Nutrition and Poultry Science

Raleigh

2003

Approved by

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DEDICATION
TO
GOD AND MY FAMILY

Biography

John Robert Upton, Jr. (Robbie) was born June 12, 1978 in Elizabeth City, NC to John R. and Mary H. Upton. Robbie has been involved with agriculture all of his life, getting his start by working on the family farm and continuing further by being active in both 4-H and FFA while growing up. In 1996, he graduated from Camden County High School in Camden, NC. His love for agriculture and science led him to study animal science at NCSU where he received a BS degree in Animal Science and a minor in Agricultural Business Management in May 2000. While at NCSU he completed internships and worked in various disciplines of animal and poultry science. After completing several research internships at the National Institute of Environment and Health Sciences (NIEHS) and NCSU, he decided to further his education with a dual Master's Degree in both Poultry Science and Nutrition under the direction of Drs. Frank Edens and Peter Ferket.

Robbie is currently employed at Embrex, Inc. as a Research Specialist in the Research and Development division. Robbie married Lauren Hix on June 29, 2002 and currently resides in Cary, NC.

Acknowledgements

I wish to extend my sincere gratitude to my graduate committee: Drs. F. W. Edens, P. R. Ferket, and C. R. Parkhurst. This fine group of scientists helped guide me through a very rewarding, yet challenging phase of my life. I am thankful to have worked with each of them; their individual and collective strengths have helped me to fulfill this goal in my life. I express sincere gratitude to Dr. Edens for his diligence to keep me on task, advice, guidance, and encouragement.

I am heavily indebted to Dr. Kamel Mahmoud for his technical work on the GSH-Px and GR enzyme assays. I learned so much from him during our short time together. He is a wonderful scientist and person. I could not have completed this degree without his help!

I want to thank Sue Mann for being such a wonderful technician and friend. I have enjoyed our endless conversations that helped me with some very difficult decisions in my life. You are a good Christian woman and I am proud to have worked with you and known you.

I thank all of my fellow graduate students in both the Poultry and Animal Science Departments for your encouragement, wisdom, and above all friendship. This entire endeavor would have been unbearable without you, your help did not go unnoticed! I extend a special thanks to Daniel Moore, Jody Smith, and Kymberly Gowdy who worked beside me so many days and gave much of their personal time to help me succeed. You guys are the greatest!

I am indebted to my parents for their unconditional sacrifices that have allowed me to achieve that which they never had the opportunity to obtain. I would like to thank my

brother, Ryan, for his love and support. It is a great pleasure to have a supportive family as I have been blessed with.

Finally I wish to convey my special gratitude to my wife, Lauren, who stood by me through the happiness and tears. I cannot express the depth of my love and appreciation for your faith and belief in me. Your unconditional love and understanding never faltered and was my inspiration as I faced the many challenges involved with this experience.

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Literature Review

Introduction

Trace elements are essential for the maintenance of health, growth, and a myriad of biochemical-physiological functions (Scott *et al.*, 1982). Selenium (Se) is an essential trace element that was discovered in 1818 by Berzelius in Sweden. No biological significance was associated with Se until it was identified as a toxic agent involved with alkali disease in the Dakota and Wyoming territories of the United States in 1856 (Franke, 1934). It was considered a dangerous element until 1957 when Schwarz and Foltz reported that Se was an essential trace element. Nutritionists and scientists then initiated extensive studies to discover the metabolic function of the element and document the consequences of its deficiency in human and animal diets. However, it was not until 1974 that Se was added as a supplement to poultry and animal diets.

Se and sulfur share similar chemical structure and it has long been postulated that they would follow similar pathways of metabolism. The discovery that plants and bacteria metabolize Se to selenocysteine and selenomethionine (SeMet) strengthened this belief (Burnell and Whatley, 1977). Organic Se, found primarily in the form of SeMet is metabolized in the same manner as methionine (Wolfram, 1999). SeMet is readily utilized as a substrate by enzymes that use methionine, and SeMet may be more available than pure methionine (Markham *et al.*, 1980). SeMet is actively transported through intestinal membranes during absorption and actively accumulated in the liver and muscle. Although inorganic Se is absorbed as a mineral, little is retained in the tissue and much of the Se is excreted *via* the feces in ruminants and urine in the non-ruminant. Very little of the inorganic Se is incorporated into body proteins (Wolfram, 1999).

In nature, Se exists in either organic or inorganic forms. Elemental Se can be reduced to the Se^{-2} oxidation state (selenide) or oxidized to the Se^{+4} (SO_3^{-2} , selenite) or Se^{+6} (SO_4^{-2} , selenate). Therefore inorganic Se can be found in the different inorganic forms of selenite, selenate, and selenide. In contrast, Se in feed ingredients is an integral part of the amino acids methionine and cysteine and exists in the Se^{-2} oxidation state. Therefore, in nature, most animals primarily receive organic Se in the form of SeMet (Combs and Combs, 1986). Plants absorb Se from the soil in the form of selenite or selenate and synthesize selenoaminoacids with SeMet representing about 50% of the Se in cereal grains (Olson and Palmer, 1976).

Absorption and Metabolism. Se absorption in the intestine is dependent upon its chemical form. McConnell and Cho (1965) examined everted intestinal sacs of hamsters and found that L-selenomethionine is transported against a gradient from the mucosal to serosal side of the intestine. The transport of L-selenomethionine was inhibited by the corresponding sulfur analogue, L-methionine. Sulfite and cystine, respectively, did not inhibit the transport of selenite and selenocystine. Spencer and Blau (1962) used ^{35}S -methionine and ^{75}Se -SeMet to show accumulation of both on the serosal side of everted hamster intestinal sacs. Chromatography was utilized to show only one gamma-emitting peak, suggesting that ^{75}Se -SeMet was not degraded in transport.

Anundi *et al.* (1984) used *in situ* intestinal loops, everted gut sacs, and isolated intestinal epithelial cells of rats to demonstrate that intestinal cells concentrate Se from selenite and that reduced glutathione (GSH) in the gut lumen plays a role in selenite absorption. When animals were pre-treated with diethylmaleate, a GSH depleting agent, and

when the agent was added to the incubation medium for intestinal cells, the rate of ^{75}Se -selenite disappearance from the luminal fluid of isolated loops reduced the accumulation of ^{75}Se from selenite in the cells. Likewise, the addition of GSH enhanced the transfer.

In vivo experiments by Reasbeck *et al.* (1981) using dogs with triple lumen gut perfusion showed that the amount of SeMet absorbed was almost twice that of selenocystine and approximately 4 times that of selenite during a 2-hour test period. This report agrees with the hypothesis that amino acid bound Se is absorbed through specific amino acid active transport mechanisms. Wolfram *et al.* (1985) investigated the intestinal absorption of selenate and selenite using *in vivo* perfusion techniques in rats. Different segments of the intestine were perfused, and the concentration dependence of ileal selenate absorption was measured. They found that selenate absorption was greatest in the ileum, where Se was absorbed by a carrier-mediated process. This absorption mechanism was not inhibited by selenite, but it can be inhibited by sulfate.

Thompson and Stewart (1975a) found absorption of Se from ^{75}Se -selenite to average 91-93% and that from ^{75}Se -SeMet to be 95-97%. In a later report, Thompson and Stewart (1975b) found that the average intestinal absorption of an oral dose of ^{75}Se -SeMet was 86% and that of ^{75}Se -selenocystine was 81%.

The Se status of an animal appears to have little effect on the intestinal absorption of selenite (Na_2SeO_3). Brown *et al.* (1972) fed rats varying levels of Se as Na_2SeO_3 (0, 0.5, 4.0 PPM in a low-Se *Torula* yeast diet) for 33 days before administering $\text{Na}_2^{75}\text{SeO}_3$, either by stomach tube or intraperitoneal injection. Absorption through the dosage range was 95-100% of the dose. These data suggests the absence of a regulatory mechanism for absorption

of selenite because absorption was not different even though a deficient level of Se was supplied for 33 days before administering the varying levels of Se.

Although Brown *et al.* (1972) reported that Se status of the animal might not affect Se absorption, Se status with respect to other elements may. Mykkanen and Humaloja (1984) reported that feeding 1000 PPM of Lead (Pb) to chickens for 3 weeks before absorption measurements of ^{75}Se -selenite resulted in a reduction in selenite transfer into the body by increasing retention in intestinal tissue. They also showed that increasing the dietary Se alleviated the inhibition. The authors hypothesized that long-term Pb exposure increased the synthesis of proteins that can bind Se, and that these binding sites may become saturated as Se intake is increased.

Whanger *et al.* (1976) demonstrated that no selenite or SeMet is absorbed from the rat stomach. They further reported that selenite and SeMet are absorbed from all segments of the small intestine, with duodenum absorption being slightly higher than from the ileum and jejunum. The specific role of the chick duodenum in the absorption of Se (selenate or selenite) was shown by Apsite *et al.* (1993). Selenite is passively absorbed in the intestine with the highest concentrations found in duodenum, liver, and kidneys (Apsite *et al.*, 1994). Pesti and Combs (1976) measured accumulation of ^{75}Se in segments of chick intestine administered $\text{Na}_2^{75}\text{SeO}_3$ and found a decrease in accumulated radioactivity from the anterior to the posterior of the gut. The importance of the upper intestine in Se absorption was verified by challenging chicks with intestinal coccidia known to infect the chick intestine at specific sites. The coccidial species that infected the duodenum and upper ileum increased the incidence and severity of exudative diathesis (ED), thus lowering Se status and reducing protein binding of ^{75}Se in the duodenum. Species that infect the posterior ileum had no effect

on manifestation of ED or on absorption of Se. Absorption of ^{75}Se from selenite, selenate, and SeMet was determined in ligated loops from duodenum, jejunum, and ileum of Se-deficient rats or rats fed selenite-supplemented diets. Se deficiency had no effect on absorption of any selenocompound in any intestinal segment. SeMet was absorbed from all segments. In comparison, selenate and selenite were most efficiently absorbed from the ileum (Vendeland *et al.*, 1992).

Absorption studies with ligated duodenal loops or oral doses indicated that high vitamin A intake (Combs, 1976) or dietary ascorbic acid (Combs and Pesti, 1976) promoted the enteric absorption of Se. Combs (1978) reported that Ethoxyquin was effective in alleviating ED when fed separately from Se. It was also effective in promoting Se utilization by increasing the plasma concentrations of the Se-containing enzyme, glutathione peroxidase (GSH-Px)(Combs, 1978).

Bioavailability. There has been much discussion concerning the bioavailability of Se. Much of this discussion revolves around the Se source. Selenite and SeMet differ in the mechanism in which they are metabolized to selenocysteine for incorporation into GSH-Px. Additionally, SeMet can be incorporated directly into body proteins and stored as such, and therefore could, inflate the bioavailability estimates based on body retention (Henry *et al.*, 1995).

Cantor and Scott (1974) examined biological availability of Se for the prevention of ED. In that study, biological availability was taken as 100% for sodium selenite. It ranged between 74% for sodium selenate to 7% for elemental Se. The biological availability of Se in feedstuffs was from 210% for lucerne meal to 60% for soybean meal and in animal feedstuffs from 25% for herring meal to 8.5% for fish solubles. Conversely, SeMet was four times

more effective than either selenite or selenocysteine with respect to prevention of pancreatic degeneration and increasing the Se concentration and pancreas weight (Cantor *et al.*, 1975).

Cantor and Scott (1974) observed that protection against ED was closely related to plasma GSH-Px. SeMet significantly increased egg production in the 3rd and 4th weeks and hatchability was significantly increased in the 2nd, 3rd, 4th, and 5th weeks by either supplement. After another three months of the basal diet fed alone or with selenite, egg production was 56 and 77% on the basal and selenite-supplemented diets, respectively, while hatchability was 10 and 90%, respectively. In prevention of ED, Se in most feeds of plant origin was highly available, from 60 to 90%, but was less than 25% available in animal products (Cantor et al, 1975). It was concluded that relative to Na₂Se₃O the retention of Se in fish meal or solubles was 43%, and relative to SeMet, 31% (Miller et al, 1972).

Hassan *et al.* (1993) observed that the bioavailability of Se in wheat and fishmeal in comparison to selenite for increasing the activity of GSH-Px was 78% and 58% respectively. Se in fishmeal was poorly available and did not prevent Se deficiency in chickens (Martello and Latshaw, 1982). Ikumo and Yoshida (1981) measured availability of Se in soybean meal, lucerne, fishmeal and SeMet, based on plasma GSH-Px and found them to be 33, 85, 82, and 92% respectively. The results of Whitacre and Latshaw (1982) clearly showed that commercially prepared fishmeal significantly decreased Se utilization and bioavailability.

Availability of Se in varying feed sources was evaluated by measuring the restoration of blood serum GSH-Px in Se-depleted chicks. The availability of the Se in capelin fishmeal was 48%, mackerel fishmeal 34%, soybean meal 17.5%, corn gluten meal 25.7%, and SeMet 78.3% (Gabrielsen and Opstvedt, 1980). However, it is important to note that there is a

problem when using GSH-Px as a marker because only 0.01 PPM of Se can maximize the enzyme's activity.

In a study that provided Se as selenite, SeCys, or SeMet, Se-deficient chicks were fed a diet containing Se at 10 µg/kg of diet. Selenite and SeCys had similar effects in promoting weight gain and preventing ED, while SeMet was less effective. In a similar study with Se provided at 60 µg/kg of diet, tissues from the chicks were analyzed for Se content. Tissue content of chicks fed selenite and SeCys were similar. SeMet fed chicks had higher Se concentrations in breast muscle and pancreas, but lower concentrations in kidney, liver, and heart (Osman and Latshaw, 1976) because muscle comprises a large percentage of the body and more Se is probably retained from feeding SeMet than from selenite.

Tissue Concentration of Selenium. The concentration of Se in organs and tissues depends on the particular tissue considered, the amount and form of Se in the diet, the length of time the diet is consumed, and the species of animal. In young animals, Se concentration can also depend on the level of dietary Se consumed by the dam. Generally, the greater the animal's Se intake, the greater the concentration in a particular tissue. The relationship is not linear. When sodium selenite is fed, the tissue concentration approaches a plateau as the Se concentration in the diet rises. The effect is not the same when SeMet is the Se source; the Se concentration continues to rise to some threshold beyond that of selenite. Latshaw (1975) reported that Se concentration of chicken liver and muscle was doubled by feeding Se in natural feedstuffs *versus* feeding the same level of Na₂SeO₃. The result was not the same when measuring blood Se concentration. Osman and Latshaw (1976) observed a great increase in pancreas Se concentration when chickens were fed SeMet as compared to an

equivalent amount of selenite. Cantor *et al* (1982) reported that SeMet greatly increased Se concentrations in pancreas, muscle, and gizzard, but not in liver when compared to selenite.

Generally, tissues ranked by Se concentration follow the order kidney>liver>pancreas>heart>skeletal muscle. This trend is observed in most species. In chickens, the general ranking of Se concentration follows the order feathers>liver>kidney> muscle>plasma (Arnold *et al.*, 1973; Echevarria *et al.*, 1988). The muscle concentration of Se is low, but due to its relatively large mass, muscle contains nearly 40% of total body Se. The liver contains about 30% and all other organs and tissues are comprised of less than 10% each (Behne and Wolters, 1983).

Excretion. Se can be excreted from the body by three major routes: the lungs, the urinary tract, and the intestinal tract. The amount and distribution of the Se eliminated depends on many factors: Se intake, form of Se, composition of the diet, as well as the physiological status of the animal.

Burk *et al.* (1972) investigated Se excretion in rats using an injected tracer dose of $\text{H}_2^{75}\text{SeO}_3$. Urinary excretion of Se was directly proportional to the dietary Se level, ranging from 6% with the low Se-supplemented diet to 67% with the high Se-supplemented diet. Fecal excretion of Se remained nearly constant at approximately 10% of the dose as the Se concentration in the diet varied. Respiratory elimination of Se was marginal and constant over the range of dietary Se levels. The authors concluded that the rats adjusted to variations in Se intake by altering the excretion of Se in the urine.

The effect of Se form on excretion in rats has been reported by many authors (Thomson *et al.*, 1975a, Thomson *et al.*, 1975b, Wright *et al.*, 1966). Thomson *et al.* (1975b) gave oral doses of ^{75}Se to rats as selenite, SeMet, or SeCys and measured urinary excretion of

Se. The authors reported excretion during the first week as 4-5% of the dose for SeMet and more than twice that amount for selenite and Se-cystine. Nahapetian *et al.* (1983) examined oral doses of each of the three forms of Se in the rat. They found that at a dose of 16 µg Se/kg body weight, urinary excretion of Se was similar for selenite and Se-Cystine, but significantly lower for SeMet. When the rats were given a dose of 1.5 mg Se/kg body weight, the urinary excretion of Se was reduced for selenite treated rats to the same percentage as that for SeMet treated rats. However, the SeCys excretion of Se remained unchanged. The decreased urinary excretion of Se from selenite was probably compensated by an increase in respiratory excretion.

Urinary excretion is a primary mechanism to decrease Se retention and maintain Se homeostasis. However, there are reports indicating Se is excreted through the feces. Thompson and Robinson (1986) reported that total recovery of Se in urine and feces were similar for both selenate Se and selenite Se. Wright and Bell (1966) examined oral doses of Se⁷⁵ in both sheep and swine. In pigs, they found that 22 % of the total oral dose of Se was recovered. Fecal excretion accounted for 15% and 7 % of the recovery as urinary excretion. In sheep, they reported 70 % of the Se⁷⁵ was recovered. The majority of the oral Se⁷⁵ was recovered in the feces (66%) as were only 4% was recovered in the urine.

Ganther *et al.* (1966) reported manipulation in the amount of volatilization of a dose of Na₂⁷⁵SeO₃ by varying the diet composition of rats. Rats fed the commercially prepared stock diet treatment volatilized about 30% of the dose within 10 hours, where rats consuming a casein-based purified diet exhaled approximately 10%. The authors postulated that with the addition of methionine, increased volatilization occurred due to greater availability of methyl groups for the formation of dimethylselenide.

Selenium Deficiency. There are a plethora of diseases associated with Se deficiency in chickens and other animals. The only clearly defined Se deficiency syndrome not related to any other deficiency is nutritional pancreatic atrophy (Combs, 1994). Only under high dietary concentrations of vitamin E (15-20 fold) or the presence of other antioxidants can the chick pancreas be protected in the absence of Se (Whitacre *et al.*, 1987). Other Se deficiencies seem to be exacerbated by vitamin E deficiencies including nutritional encephalomalacia (Century and Hurwitt, 1964; Combs and Hady, 1991) and ED (Noguchi *et al.*, 1973; Barthlomew *et al.*, 1998). Therefore, all three of these Se deficiency diseases are related in some form to dietary antioxidant status.

Se deficiency is also associated with impaired development of immunocompetence, and reproductive failure in breeding chickens (Combs and Combs, 1984). Marsh *et al.* (1981) demonstrated that Se was required for normal development of immunocompetence in the chick by feeding diets deficient in either Se or Vitamin E for the first 2 weeks after hatching. The chicks exhibited impaired humoral responses to sheep red blood cells. However, Se and vitamin E appeared to be mutually replaceable for this function by 3 weeks of age.

Combs and Scott (1977) showed hatchability of eggs was reduced by a low-Se diet and was further depressed by peroxidized fat. The hatchability was restored to normal by supplementation of Se and Vitamin E. Cantor *et al.* (1978) showed that Se is required in breeder turkey diets for optimum hatchability and viability of offspring. Egg production and fertility were maintained at 77% and 92%, respectively, by diets supplemented with Se and were reduced to 56% and nearly 0%, respectively, when fed the basal diet that was low in Se (Cantor and Scott, 1974).

Selenium Toxicity. Se has been shown to be toxic to poultry when used in high doses. It usually is only seen when the dose exceeds at least 10-fold the physiological requirement. However, Arnold et al. (1972; 1973) reported that the effects of an accidental addition of as much as 80 times the requirement could be quickly rectified. Although there are conflicting reports, Se doses at lower than 3-5 mg/kg of feed are usually not involved with toxicity.

Moksnes (1983) fed white leghorn chickens a basal diet containing Se at 0.30 mg/kg. The diets were supplemented with 0, 0.1, 0.5, 1.0, 3.0, and 6.0 mg/kg of Se in the form of SeMet for 18 weeks and no toxic effects observed even at the highest intake of Se (Moksnes, 1983).

Kaantee *et al.*, (1982) reported no adverse effect on parent birds or on hatching of eggs when 40 Leghorn hens and five cockerels were fed diets containing 0.14 to 0.85 mg/kg of Se. When Se as sodium selenate was supplemented in feed from 0.1 to 9 mg/kg, hatchability of fertile eggs was significantly decreased by 5 mg/kg Se and higher (Kaantee *et al.*, 1982). Egg weights were decreased by 7 mg/kg and higher and egg production was decreased only by 9 mg/kg (Ort and Latshaw, 1978). Todorovic *et al.* (1999) fed day old chickens basal diets supplemented with 0, 2, 10, 20, and 30 mg Se/kg as sodium selenite for six weeks. The lowest levels at which dietary Se caused reduction in daily gain was 5 mg/kg. Diets supplemented with 10, 15, and 20 mg Se/kg produced 24.5, 62.7, and 96.6% reductions in daily gain, respectively. Mortality was reported as 26.7, 60, and 80% when diets were fed with 15, 20, and 30 mg Se/kg respectively (Todorovic *et al.* (1999).

Salyi *et al.* (1993) reported the oral LD₅₀ of sodium selenite to be 9.7 mg/kg of body mass for chickens. In another experiment the LD₅₀ for chickens was reported as 24.6 mg Se/kg of body weight (Tishkov and Voitov, 1989). The authors also reported species-

specific differences in susceptibility to Se toxicity indicating the LD₅₀ for turkey poult and ducks to be 13.5 and 64 mg/kg of body weight respectively. In turkey poult the minimum toxic dose rate of sodium selenite was 0.9 mg/kg body weight, as with broiler chicks it was found to be 1.7 mg/kg and 9.4 mg/kg in ducks (Tishkov and Voitov, 1989). Akulov *et al* (1972) found the LD₅₀ to be 33.4 mg/kg body weight and the maximum tolerated dose was 15 mg/kg body weight in laying hens.

Se toxicity produces characteristic signs such as growth depression, reduced egg production, anemia, and stiffness of the tibiotarsal joints (Soffietti *et al.*, 1993). Qi *et al.* (1992) fed chicks toxic doses of organic Se (8-13 PPM in Se-enriched corn) and found the pathological changes were characterized by local necrosis in the liver, myocardial degeneration and convoluted tubule necrosis in kidneys. When Se poisoning was experimentally induced by Salyi *et al* (1993), dyspnea, diarrhea, and weakness were observed within a short time.

It is important to note that the molecular mechanisms of Se toxicity are not well defined. However, considerable evidence has accumulated, suggesting that oxidative stress may be the main molecular mechanism of selenosis (Raisbeck, 2000). A reaction between selenite and GSH with production of free radicals could explain the pro-oxidant properties of selenite. Raisbeck (2000) reported that the primary targets of Se toxicity in food animals are the cardiovascular, gastrointestinal, and hematopoietic systems.

Selenoproteins. The physiological roles of Se began with groundbreaking work by Rotruck *et al.* (1973) that identified Se as a stoichiometric component of glutathione peroxidase. Soon thereafter in the mid-1980's, more selenoproteins were discovered and Se biochemistry began to broaden.

Glutathione Peroxidases. The first selenoprotein discovered was glutathione peroxidase (GSH). Ursini *et al* (1995) described four structurally and genetically different forms of Se-containing GSH-Px that exist in different tissues or parts of the cell. The most abundant selenoprotein in mammalian tissues is the cytosolic GSH-Px (cGSH-Px). This classical form of GSH-Px efficiently metabolizes hydrogen peroxide as well as unesterified fatty acid hydroperoxides in conjunction with GSH, the pentose phosphate cycle, and glutathione reductase (GR) (Wolffram, 1999). cGSH-Px is a tetrameric protein with four identical subunits, each containing one Se atom (Sunde, 1993). The second cytosolic GSH-Px was identified in the gastrointestinal tract cells and is referred to as GSH-Px-GI. The enzymatic properties of this form are practically the same as those of cytosolic GSH-Px. The physical properties of the two are also similar, with the activity of both depending on Se supply (Chu et al., 1993). GSH-Px-GI activity was reported in both the villus and crypt regions of rat mucosal epithelium, and its activity nearly equaled that of the classical GSH-Px throughout the small intestine and colorectal segments (Esworthy et al., 1998). The third form, which is extracellular, is present in the plasma and denoted as pGSH-Px (Daniels, 1996).

Phospholipid hydroperoxide GSH-Px (GSH-Px-PH), a membrane-bound form of GSH-Px, plays an important role in the interaction of vitamin E and Se. It has been postulated that GSH-Px-PH along with vitamin E acts as a chain-breaking antioxidant to protect phospholipid membranes. GSH-Px-PH may also be involved in regulation of leukotriene biosynthesis. This enzyme is preferentially expressed in endocrine and reproductive tissues and has been shown to play a major role in male reproduction (Kohrle *et*

al., 2000). Ursini et al. (1999) found that in the testis, the GSH-Px-PH acts as a powerful antioxidant in the developing spermatids and spermatozoa. GSH-Px-PH is distinguished from classical GSH-Px as it is active in monomeric form and has a different amino acid composition (Sunde, 1993).

Thioredoxin reductase. Stabilizing disulfides are abundant on cell surfaces and extracellular proteins due to the oxidizing nature of the environment. The inside of the cell is maintained in a very reduced state where intracellular proteins contain many free sulfhydryl groups (-SH) and very few disulfides. Thioredoxin is the major disulfide reductase that maintains the proteins in a reduced state. Thioredoxin and GSH work together to keep intracellular redox potential low and maintains free -SH groups (Arner and Holmgren, 2000).

Thioredoxin is able to transfer reducing power to cellular proteins by the enzyme thioredoxin reductase. Thioredoxin reductase (TR) uses electrons from NADPH to reduce oxidized thioredoxin. All mammalian TRs are Se-dependent flavoproteins (Tamura and Stadtman, 1996).

The physiological function of the thioredoxin system is being studied extensively. Mustacich and Powis (2000) reported that thioredoxins are high capacity electron donors for reductive enzymes including ribonucleotide reductase, thioredoxin peroxidase, and through thiol/disulfide exchange reduce key cysteine residues in transcription factors that increase binding to DNA and alters gene transcription. Thioredoxins also function as cell growth factors and have the ability to inhibit apoptosis. Arner and Holmgren (2000) report that TR reduces hydroperoxides, ascorbate and selenite. It has been suggested that ascorbate may be the biochemical link between vitamin E and Se because it has been shown to recycle tocopheroxyl to tocopherol *in vitro* (Burk and Hill, 1999).

Selenoprotein P. Selenoprotein P, along with plasma/extracellular GSH-Px, is the only other known extracellular selenoprotein. Selenoprotein P is unique among the selenoproteins because it contains multiple selenocysteine residues. Selenoprotein P has been isolated from various tissues of the mouse, rat, human, and bovine (Burk and Hill, 1999). Selenoprotein P constitutes a large portion of the Se levels in plasma. It has been shown that more than 60% of plasma Se was found in selenoprotein P in rats (Burk and Hill, 1999) while only 50% in humans (Xia *et al.*, 1992).

A specific biochemical function has not been described for selenoprotein P, but both extracellular antioxidant and Se transport roles have been suggested. Burk *et al.* (1997) reported the association of selenoprotein P with endothelial cells in liver, kidney, and brain of rats. Selenoprotein P binds heparin proteoglycans on cells and in the interstitial matrix since it is secreted into plasma by the liver and into interstitial spaces by cells in virtually all tissues. Burk and Hill (1993) suggest that selenoprotein P functions to protect endothelial cells against oxidants.

Selenoprotein P is responsive to dietary Se and is reduced to 5-10% of control levels in rats fed deficient diets. Selenoprotein P has a higher priority for synthesis and declines less rapidly when compared to GSH-Px when animals are fed diets that are limiting in Se (Yang *et al.*, 1989; Burk and Hill, 1993).

Selenoprotein W. Selenoprotein W has been isolated from the mouse, rat, monkey, human, and sheep (Burk and Hill, 1993). Although the physiological function is not understood, it is known to be located in the cell cytosol and the tissue occurrence appears to

be species dependent (Yeh, *et al.*, 1997a). The highest concentration of selenoprotein W is found in the heart and muscle of sheep. Thus, its relationship to the etiology of white muscle in sheep has been studied. Yeh *et al.* (1997a) studied sheep fed diets that were deficient in Se or supplemented with selenite (3mg/kg) and investigated selenoprotein W content. All tissue levels of selenoprotein W were sensitive to dietary Se content except the brain. The sheep fed deficient diets had brain tissue levels of selenoprotein W at the same level of those fed the supplemented diets. However, there was a 53% reduction in total brain Se content and a 30% reduction in GSH-Px activity. These results indicated that the regulation of selenoprotein W synthesis in the brain differed from other tissues. In another report by the same authors, it was shown that rat tissues differed in rates of increase in selenoprotein W content in response to graded increases in dietary Se. The report also indicated that there was little correlation with tissue GSH-Px activity or tissue Se content (Yeh *et al.* 1997b).

Iodothyronine Deiodinases. Iodothyronine Deiodinases (ID) are the second largest group of selenoproteins. The three deiodinases (Type I, II, and III ID) control the local availability and concentration of the active thyroid hormone, 3,3',5-triiodothyronine (T_3). These enzymes catalyze the conversion of thyroxine (T_4) to T_3 (Type I and II ID) or the deiodination of T_4 and T_3 to non-active metabolites (Type III ID). These three isoenzymes are encoded by different genes and have tissue and development-specific patterns of expression and regulation (Kohrle *et al.*, 2000). In general, ID is ranked higher in priority for available Se supply than is cytosolic GSH-Px and was similar in ranking to that for GSH-Px-PH and selenoprotein P (Kohrle, 2000). Because thyroid hormone controls growth, development, differentiation and many metabolic reactions, Se is believed to be involved in regulation of those functions as well.

In Se-deficient animals, activity of ID I is low. This results in plasma T_4 increase while T_3 is decreased. A role for Se in type I deiodinase and thyroid hormone metabolism has many implications. The reduced activity of thyroid hormone explains why Se-deficient animals grow more slowly as effects of this hormone are mainly anabolic (Arthur, 1993). Reduced ID I activity in the pituitary is associated with lower levels of growth hormone in Se-deficient animals (MacPherson, 1994).

Thyroid hormone activity is a key factor in animal tolerance to cold stress. A well-known thyroid hormone function is the heat-producing increase in oxygen consumption of tissues in response to cold temperatures. Involvement of thyroid hormones in feathering has long been reported. The active T_3 is known to be intimately involved in feather development. During periods of feather growth, basal metabolic rate increases to provide energy for feather production and to keep the bird warm. Thyroid hormone levels increase and in response the bird increases heat production. If Se is limiting, T_3 levels might be expected to be lower. As feather cover increases, the basal metabolic rate falls and heat production is diminished because oxidative metabolism decreases (Edens, 2000).

Glutathione Reductase. Glutathione Reductase (GR) is a ubiquitous enzyme that catalyses the reduction of oxidized glutathione (GSSG) to GSH in the presence of reduced NADPH. GR is essential for the GSH redox cycle that maintains adequate levels of reduced cellular GSH, the predominant form in the tissues, which serves as an antioxidant reacting with free radicals and organic peroxides (Rall and Lehninger, 1952). It is generally accepted that GR is largely responsible for the maintenance of intracellular glutathione in the reduced form. However, the enzyme also functions intracellularly in the reduction of mixed disulphides of GSH and protein.

GR is an important enzyme associated with the hexose monophosphate pathway in erythrocytes (Harris and Kellermeyer, 1970). Research has shown GR to be a flavin enzyme with flavin dinucleotide (FAD) serving as an apparent prosthetic group (Scott et al., 1963; Buzard and Kopko, 1963; Staal et al., 1969; Staal and Veeger, 1969).

The Se-dependent GSH-Px system, which is vital for the protection against tissue peroxidation, requires the following nutrients: Se as a component of the GSH-Px, the sulfur amino acids in the form of cysteine in GSH, riboflavin in FAD for GR activity and niacin as a component of reduced NADPH.

Both GSH-Px and GR are involved in the cycling of glutathione. GSH-Px oxidizes glutathione while reducing toxic endogenous and exogenous peroxides while GR restores the reduced glutathione status. GR activity may be associated with the requirement for the accumulation of oxidized GSH, which is the product of the reaction catalyzed by GSH-Px. (Pablos et al., 1998).

Studies with humans (Beutler, 1969; Bamji, 1969; Tillotson and Baker, 1972) and rats (Glatzle et al., 1968; Srivastava and Beutler, 1970; Buzard and Srivastava, 1970) demonstrated that measurement of erythrocyte GR (EGR) activity would be a useful and sensitive procedure for evaluating the riboflavin status of individual subjects or population groups. The EGR activity is altered in vivo by dietary riboflavin and in vitro by FAD. The degree of in vitro stimulation or EGR activity is dependent on the FAD saturation of the apoprotein, which in turn is dependent on the availability of riboflavin. The action of GR is also critically dependent on an adequate supply of NADPH (Godin and Garnett, 1992).

Rutz et al. (1988) reported that whole blood GR was reduced in broilers receiving low riboflavin diets. These authors later reported a significant interaction of Se and riboflavin on

erythrocyte GR activity coefficient (Rutz et al., 1989). Both Se and riboflavin decreased the coefficient. Brady et al. (1979) reported that erythrocyte GR activity, but not hepatic and muscle GR activity, was increased by riboflavin supplementation in baby pigs. Parsons et al. (1985) reported the percentage active GR declined rapidly when pigs were placed on a riboflavin-unsupplemented diet and was lower than that of riboflavin-supplemented pigs.

There are large differences in the activity of both plasma and erythrocyte activity among species reported in the literature. Unlike erythrocyte, plasma GR is not significantly stimulated by the addition of FAD. In species with high plasma GR activity the determination of whole blood FAD activity coefficients may not be a good index of riboflavin status (Board and Peter, 1976).

Immunology. Se deficiency has been reported to decrease both cellular and humoral immune function in man and laboratory animals (Combs and Combs, 1986). The knowledge of specific mechanisms in livestock is less detailed than in laboratory animals although the increase in susceptibility to disease in deficient livestock is well documented (Maas, 1998). Sordillo *et al.*, (1997) reported that Se deficiency is an established risk factor in mastitis incidence and has been correlated with decreased bactericidal activity of neutrophils and the severity of mastitis infection.

Organic vs. Inorganic Se For many years it has been recognized that the selenoaminoacids: SeMet, selenocysteine, and selenocystine, are the sources of naturally occurring Se in plant based and meat based feed ingredients (Burk, 1976; Levander 1986; Cai et al., 1995). These selenoaminoacids are bound in protein as SeMet and selenocystine and constitute 50-80 % of total Se in plants and grains (Butler and Peterson, 1967) and in

Sel-Plex™(Alltech, Inc., Nicholasville, KY), an organic Se-enriched yeast (Kelly and Power, 1995).

SeMet cannot be directly synthesized from selenite or selenate by animals (Cummins and Martin, 1967; Sunde, 1990). However, selenocysteine can be found in the body of animals fed inorganic Se as selenite and selenate. The presence of selenocysteine is due to the synthesis of glutathione and other selenoproteins, in which the selenocysteine is incorporated. The Se in selenocysteine is incorporated co-translationally using selenide and serine as precursors. The synthesis of selenocysteine involves a unique process where selenide is phosphorylated under the influence of selenophosphate synthetase to selenophosphate. The selenophosphate is made available to a unique seryl-tRNA^{SEC} that is recognized by selenocysteine synthetase. The selenocysteine synthetase converts seryl-tRNA^{SEC} to selenocysteyl-tRNA^{SEC} that allows insertion of selenocysteine into a peptide chain (Amberg et al., 1996). The base triplet UGA that ordinarily functions as a stop codon encodes this process of selenocysteine insertion at its appropriate site in the peptide. The selenocysteine insertion also requires a specific mRNA, an elongation factor, GTP, and the selenocysteine insertion sequence that all interact at the ribosome to read the UGA selenocysteine codon (Low and Berry, 1996).

SeMet is converted to selenocysteine via the enzyme cystothionase (Esaki et al., 1981). Poultry cannot synthesize cysteine *de novo* so SeMet is required if there is a conversion of SeMet to selenocysteine (Cummins and Martin, 1967; Esaki et al., 1981). Selenocysteine can be substituted for cysteine in many proteins, but it is not incorporated directly into selenoproteins (Sunde, 1990; Daniels, 1996). To allow selenocysteine to be incorporated into specific selenoproteins, there is a requirement for selenocysteine-β-lyase

reaction with free selenocysteine to release selenide in the presence of reducing agents (Sunde, 1990; Burk, 1991). Next, Selenocysteyl-tRNA[Ser]^{SEC}, which recognizes the specific UGA stop codons in the selenoprotein-mRNA, inserts the new, co-translationally synthesized selenocysteine into the specific selenoprotein (Burke, 1991). Therefore, organic Se must be converted from its original organic to inorganic form and then back to the organic form to fulfill its biological function (Arthur, 1997). This conversion is crucial with regard to the synthesis of selenoproteins because it has been reported that 30 to 80% of the Se in the body may be selenocysteine (Hawks et al., 1985).

In instances where SeMet is supplemented at high levels, it can be demonstrated that 40 to 50% of total body Se as SeMet can be found in muscle (Daniels, 1996). Selenocysteine is the pivotal amino acid in the synthesis of Se-dependent cytosolic glutathione peroxidase (Rotruck et al., 1973), but only about 30 % of the body's Se is incorporated into 30 to 100 other selenoproteins in mammals (Burk and Hill, 1993).

Positive Responses to Sel-Plex by Broiler Chickens. Sel-PlexTM, a selenized yeast product by Alltech, Inc (Nicholasville, KY) was recently approved in the United States as a source of organic Se for broiler diets. Sel-PlexTM provides a cocktail of Se compounds (Kelly and Power, 1995) but SeMet is the primary form of Se in Sel-PlexTM. Kelly and Power (1995) reports that the organic Se profile in Sel-PlexTM is similar to the organic Se profile found in plants and grains. Organic Se in Sel-PlexTM is readily available and is actively absorbed from the intestine *via* the Na⁺-dependent neutral amino acid pathway, where selenite is passively absorbed (Schrauzer, 2000).

In numerous studies, Sel-PlexTM has been proven to be superior to selenite as a nutritional source of Se. Edens et al. (2000) reported the induction of feathering causing sex-

linked slow feathering broiler males and normal feather females to increase feather development when fed diets supplemented with Sel-Plex™. Sel-Plex™ has been shown to be superior in tissue accumulation and retention in broilers (Norheim and Moksnes, 1985) and in reducing drip loss from breast meat (Edens 1996, Naylor et al., 2000), and reduce the incidence of pale soft, exudative meat from broilers (Edens, in review). The many positive responses to the inclusion of Sel-Plex in the diet have fueled interest in the use of Sel-Plex™ in all sectors of the poultry industry.

Selenium functions as an antioxidant. Most animals, plants, and microorganisms rely on oxygen for the efficient production of energy. However, the high oxygen concentration in the atmosphere is potentially toxic for living organisms because free radicals derived from oxygen can damage many types of biological molecules. The presence of natural antioxidants in living organisms enable the survival in an oxygen-rich environment (Halliwell, 1994).

The formation of free radicals is a pathobiochemical mechanism involved in the initiation or progression of various diseases (Hogg, 1998). In livestock production, free radical generation and lipid peroxidation are responsible for the development of various diseases, decreases in animal productivity, and product quality (Hurley and Doane, 1989; Weiss, 1998; McDowell, 2000).

A variety of different stress conditions are associated with the over-production of free radicals and thus cause a disturbance in the balance of prooxidant-antioxidant leading to the potential for tissue damage (Jaeschke, 1995). Stress conditions are usually divided into three main categories: nutritional stress, environmental conditions, and internal stress. All of these conditions stimulate free radical generation by decreasing the coupling of oxidation and

phosphorylation in the mitochondria that results in an increased electron leakage and over production of superoxide radical (Dalton et al., 1999).

Once free radical production exceeds the antioxidant systems capacity to neutralize, lipid peroxidation causes damage to unsaturated lipids in cell membranes, amino acids in proteins, and nucleotides in DNA. Thus resulting in membrane and cell integrity disruption. This inevitably results in decreased productive and reproductive performance.

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Manuscript I: Effect of Selenium Supplementation on Performance and Production Parameters in Broiler Chickens

ABSTRACT In August 2000, organic selenium (Se) in a Se yeast (Sel-Plex™, Alltech, Inc., Nicholasville, KY 40356), which contained high levels of selenomethionine, was approved by the United States Food and Drug Administration as a source of Se supplementation for broiler chickens. The previously reported positive responses to the presence of selenomethionine in Se yeast supplemented feed have increased the interest in use of organic Se in all phases of poultry production. Thus, a series of experiments to test the influences of Se yeast on performance parameters were conducted using broiler males (Arbor Acres X Arbor Acres) in floor pens. A completely randomized experimental design incorporated four treatment groups for three diets: Starter- 3177 kcal ME/kg, 22.5% crude protein (CP); Grower- 3168 kcal ME/kg, 19.5% CP; Finisher- 3160 kcal ME/kg, 17.4% CP. Treatment groups consisted of (1) no Se, (2) sodium selenite (0.2 ppm), (3) Se yeast (0.2 ppm, Sel-Plex™, Alltech), and (4) the combination of selenite (0.1 ppm) + Sel-Plex™ (0.1 ppm) for each diet. Body weights (BW), feed conversions (FCR), cut up parts yield as a percentage of carcass weight, drip loss from breast meat, and serum thyroid hormones were measured up to 42 days of age. At 6 wk of age, BW of broilers fed the Se yeast treatment were increased as compared to those in the no supplemental Se or sodium selenite treatment groups. The combination of sodium selenite and Se yeast was no more effective than selenized yeast alone on BW. FCR were improved by all Se sources, with the Se yeast and Se yeast + sodium selenite treatments superior to sodium selenite only fed birds. On a percentage of carcass weight, yields of viscera, feet, and neck were higher in Se-yeast treated birds. Yields of leg and *pectoralis major* as percentages of carcass weight were increased and decreased,

respectively in Se yeast fed birds. There was an increase in breast meat drip loss when birds were fed sodium selenite as compared to both the Se yeast and no supplemental treatments, suggesting that sodium selenite supplementation may be associated with oxidative tissue damage that results in increased moisture loss from processed breast meat. The serum T_4 levels were higher in birds within the no supplemental Se treatment group as compared to those supplemented with sodium selenite or Se yeast. The ratios between serum T_4 and T_3 indicate that Se yeast treatment facilitated the conversion of T_4 to T_3 , possibly extra-thyroidally in the liver. The results from these studies suggest that selenomethionine from selenized yeast may be a superior form of Se in poultry for optimal production performance in fast growing, high yielding broiler chickens.

Key Words: selenium, selenomethionine, sodium selenite, thyroid hormone

INTRODUCTION

In 1817, Berzelius, the Swedish chemist, discovered Se and determined that it was an atomic element closely associated with sulfur and tellurium. Unfortunately, a biological significance of this element was not recognized until it was identified as the toxic agent linked with “alkali disease”, which is now referred to as selenosis, in the Dakota and Wyoming territories in the United States (Franke, 1934). Se was considered to be a dangerous element until Schwarz and Foltz (1957) found Se to be an essential dietary nutrient that prevented nutritional liver necrosis in rats. In the same year, prevention of exudative diathesis by Se was reported in chicks (Patterson et al, 1957). Nutritionists soon initiated extensive studies to discover the metabolic function(s) of Se and document the

consequences of Se deficiency in human and animal diets. Se deficiency manifests itself in poultry in many diseases and dysfunctions, including liver necrosis, muscular dystrophy, microangiopathy, exudative diathesis, pancreatic fibrosis, poor feathering, immune deficiency, reduced hatchability, and many others (Edens, 1996).

The previously identified signs of Se deficiency in chickens have been related to the role of Se in antioxidant protection *via* the enzyme glutathione peroxidase (GSH-Px). For many years, this was the only known role of Se in any species. More recently, a number of selenoproteins have been identified that both expand our knowledge of the physiological function of Se and aid in explaining other signs of Se deficiency (Arthur, 1997; Jacques, 2001).

Due to the similarities between Se and sulfur, it has been a long held belief that Se would follow similar pathways in its metabolism as sulfur. Burnell and Whatley (1977) strengthened this concept when they reported that plants and bacteria metabolize Se to the organic selenomethionine and selenocysteine. Selenomethionine is readily utilized as a substrate by enzymes that use methionine, and selenomethionine may even be more available than pure methionine (Markham et al., 1980).

It has been accepted for many years that the selenoamino acids: selenomethionine, selenocysteine, and selenocystine, are the primary sources of naturally occurring Se in plant based (Burk, 1976) and meat based (Levander 1986; Cai et al., 1995) feed ingredients. The selenoaminoacids are bound in protein, predominantly as selenomethionine and selenocysteine and can constitute up to 80% of the total Se in plants, grains (Butler and Peterson, 1967) and in Sel-Plex™(Alltech, Inc., Nicholasville, KY 40356), an organic Se-enriched yeast (Kelly and Power, 1995).

In June 2000, the United States FDA approved selenized yeast (Sel-Plex™) as an organic Se source for the use in broiler chickens. Conventionally, Se has been supplemented in animal feeds in the inorganic form as sodium selenite. Sel-Plex™ affords a mixture of organic Se compounds (Kelly and Power, 1995), but the primary form is selenomethionine (~50%) in the yeast cellular protein component. The organic Se in Sel-Plex™ is readily available and is actively absorbed from the intestine *via* the Na⁺-dependent neutral amino acid pathway, while selenite is passively absorbed (Schrauzer, 2000). Furthermore, it has been reported that Sel-Plex™ is superior to sodium selenite in conditions related to feather development, causing sex-linked slow feathering broiler males and normal feathering females to increase feather development (Edens *et al.*, 2000), in tissue accumulation and retention in broilers (Norheim and Moksnes, 1985), and in reducing drip loss from breast meat (Edens, 1996; Downs *et al.*, 1999; Naylor *et al.*, 2000). The above-mentioned positive responses to Sel-Plex™ supplementation have fueled interest in the use of Sel-Plex™ in all sectors of the poultry industry. Therefore, it is crucial to not only continue the comparison of the influence of Se sources on production performance of high yielding broilers but to also elucidate the influence of organic Se on the physiological and biochemical status of the broiler chicken.

The objectives of these studies were 1) to determine the influence of Se source on production performance of broilers, 2) determine the influence of Se source on cut-up yields from carcasses of broilers, 3) determine the influence of Se source on drip loss from breast meat of broilers, and 4) determine the influence of Se source of serum thyroid hormone levels in broilers.

MATERIALS AND METHODS

Animal Welfare. This project was approved and conducted under the supervision of the North Carolina State University Animal Care and Use Committee which has adopted Animal Care and Use Guidelines governing all animal use in experimental procedures.

Animals and Experimental Treatments. A series of experiments were conducted to determine the influence of Sel-Plex™ on production performance of high yielding Arbor Acres X Arbor Acres cockerels. The studies were conducted in conventional broiler production facilities. Forty birds were placed in 3.5 X 12-foot floor pens. The pens contained 2 feeders and 1 plasson drinker and fresh pine shaving litter.

Diets. The experimental diets consisted of the North Carolina Agricultural Research Service standard starter, grower, and finisher diets. The starter diet was fed from 1-16 days of age and consisted of 3177 kcal/kg ME and 22.5% CP. The grower diet was fed from 16-35 days of age and was 3168 kcal/kg and 19.5%CP. The finisher diet was formulated to contain an energy value of 3160 kcal/kg and 17.5% CP and fed from 35-42 days of age. The diets were supplemented with organic Se yeast (SP, Sel-Plex™, Alltech, Inc.) or sodium selenite (SEL) at 0.2 mg Se/kg of feed. The background levels of Se were analyzed to be 0.28 mg/kg, 0.28 mg/kg, and 0.24 mg/kg in the starter, grower, and finisher diets, respectively. The feed was adjusted to allow per bird consumption an average of 2 lbs. of starter, 5 lbs. of grower, and 2 lbs. of finisher. The experiment utilized a 2 x 2 factorially arranged completely randomized statistical design. Se was supplemented to the diets as follows: Diet 1: no supplemental Se; Diet 2: SEL at 0.2 ppm Se; Diet 3: SP at 0.2 ppm Se; Diet 4: SEL (0.1 ppm Se) + SP (0.1 ppm Se).

Parameters. BW were obtained at 2, 4, and 6 weeks of age and FCR and mortality was calculated through six weeks of age. Forty birds per treatment were taken for cut up parts yield as a percentage of carcass weight and twenty birds per treatment were used to calculate drip loss from breast meat at six weeks of age. The *pectoralis major* was dissected and chilled in ice water for 4 hours. The muscle was blotted dry and a one-inch cube was cut and weighed. The cube was suspended from a stainless steel hook attached to the weight-tared lid of a weight-tared jar. The jar/lid unit was closed and was weighed and recorded on a 24-hour basis. By gravimetric analysis, drip loss was determined by weight changes in the weight-tared jar without the lid. During the processing of carcasses, a determination concerning the Pale Soft Exudative (PSE) status of the breast meat was made. Pale, friable breast muscle was considered affected by the PSE condition. Frequency of PSE was also determined among the treatments.

At 2, 4, and 6 weeks of age, venous blood samples were collected from 10 birds per treatment. Blood was drawn into syringes without anticoagulant and introduced into serum separation tubes¹ and allowed to clot. The clotted blood was allowed to sit for six hours at room temperature. The samples were then centrifuged at 400 X G for 30 minutes. Serum was decanted and frozen at – 40°C until analyzed for thyroid hormones. The serum thyroid hormones, thyroxin and triiodothyronine, were determined with the non-radioactive Morningstar ELISA assay².

Statistical Analysis. Data from the experiment were subjected to statistical analysis using the General Linear Model procedures of the Statistical Analysis System (SAS, 1996).

¹ Beckton Dickinson, Franklin Lakes, NJ 07417

² Morningstar Diagnostics, Inc., Naperville, IL 60563

RESULTS

Production Performance. Performance was improved in broilers fed SP when compared to birds fed no supplemental Se or SEL (Table 1). Body weight at 42 days was increased in SP fed broilers as compared to those in the no supplemental Se or SEL treatment groups. The combination of SEL and SP did not improve body weight over the SP group. Feed conversions (FCR) were improved by all Se sources, with the SP and SP + SEL treatments superior to SEL only.

Body weight improvements were apparent in the SP fed chickens as early as 2 weeks of age, but were improved significantly at 4 and 6 weeks compared to SEL and no supplemental Se groups. Feed conversions were improved by both Se sources when compared to FCR for broilers in the no supplemental Se treatment group.

Pale, Soft, and Exudative Breast Meat. The data in Figure 1 demonstrate that SP was more efficient than SEL in reducing the frequency of PSE breast meat from chickens reared in the spring and summer. The lower level of SP (0.1 ppm Se) was equivalent to or better than the higher inclusion (0.3 ppm Se) of SEL in reducing the frequency of PSE meat.

Cut-Up Yields of High-Yielding Broiler Cockerels. On a percentage of carcass weight, yields of visceral, feet and neck were higher in SP treated birds (Table 2). Yields of leg and *pectoralis major* as percentages of carcass weight, were increased and decreased slightly, respectively, in SP treated birds. Additionally, there was a trend toward higher yield of thigh meat from the birds supplemented with SP (Table 3).

Breast Meat Drip Loss. There was an increase in breast meat drip loss when birds were fed SEL when compared to both the SP and no supplemental Se treatments (Figure 1).

Serum Thyroid Hormones. The serum thyroxin (T_4) levels were higher in birds within the no supplemental Se treatment when compared to those supplemented with SEL or SP (Figure 2). No differences in serum T_4 were found due to Se source from 2 to 6 weeks of age. Serum triiodothyronine (T_3) levels were lower in broilers given no supplemental Se compared to broilers supplemented with either SEL or SP (Figure 3). Serum T_3 levels were marginally lower in SEL treated birds, than those broilers fed SP. The ratios between serum T_4 and T_3 are shown in (Figure 4).

DISCUSSION

The National Research Council (1994) reports that the Se requirement to support normal broiler chickens is 0.1 ppm. The background levels of Se in the basal diets averaged 0.26 ppm during the course of these studies. In this case, the background level should have met the Se requirements of the broiler chickens studied. However, the data propose that there may be an additional requirement for Se by the faster growing, higher yielding broilers that are commonly grown today and that Se supplemented in the organic form may be more useful than inorganic forms of Se. The data illustrate that SEL broilers lagged behind the SP birds until 6 weeks of age, apparently because they were slower to feather during the first 5 weeks of age (Edens et al., 2000). Granted, background levels of Se were sufficient to maintain good performance by the broilers in this study, but additional Se appeared to be necessary to optimize growth with birds given SP showing a greater response.

The FCR data imply that an additional requirement for Se may be necessary for the modern broiler chicken. Additionally, SP appeared to be superior to SEL in satisfying the additional requirement.

Pale, soft, exudative (PSE) meat is a rising problem among high yielding meat animals. It has been studied comprehensively in swine and turkeys (Ferket and Foegeding, 1994). These authors reported that high levels of vitamin E supplementation in the diet could control a great amount of the PSE problem in turkeys. This report suggests that there may be an oxidative problem associated with post-mortem development of PSE meat (Ferket and Foegeding, 1994). In more recent work with broiler chickens, Van Laack et al. (2000) reported that PSE develops when there is accelerated post-mortem glycolysis and rapidly decreasing pH in meat that is still warm. These conditions yield meat that is pale with decreased water holding capacity and poor texture (Ferket and Foegeding, 1994).

Our data suggest that there may be an oxidative stress that contributes to the development of PSE meat. The SEL-fed broilers were found to be more susceptible to PSE development than SP fed broilers. These observations imply that superior tissue retention of organic Se in SP and steady state release of organic Se for incorporation into the glutathione/glutathione peroxidase antioxidant system was important in the reduction of PSE condition in broiler chicken meat.

In this study, cut-up yields of carcasses were influenced by Se source. A significant increase ($P < 0.05$) in the yields of viscera, feet and neck as a percentage of carcass weight were found in SP fed animals. A large visceral weight may be due to a healthier gastrointestinal tract and therefore less gut mucosal degradation. The result of this condition could render more efficient utilization of feed. This result was reported in several other studies (Naylor *et al.*, 2000; Roch *et al.*, 2000) where improved FCR was reported. The increase in yields of feet and neck and a trend toward a larger head may also reflect improved growth in the SP treated broilers. Feeding SP increased leg and thigh yields ($P < 0.05$),

confirming earlier observations (Edens, 1996; Naylor *et al.*, 2000). On the contrary, feeding SP also yielded negative results. There were reduced yields of *pectoralis major* muscle in the breast of male broilers fed diets supplemented with SP. Edens (1996) first reported this negative effect of reduced *pectoralis major*.

The drip loss data presented suggest that SEL Se may be associated with an oxidative process that promotes postmortem-development of compromised cell membranes and facilitates increased moisture loss from processed breast meat. This data is in agreement with Mahan's (1999) observations with swine and Downs et al.'s (1999) in broiler chickens. The data from this study show that there was less drip loss observed in breast meat from broilers fed organic Se than those fed inorganic Se. This information is applicable to the poultry industry because in poultry processing facilities, the carcass is chilled in a hypotonic ice-water bath. The flesh of the carcass usually absorbs the water from this ice bath due to the fact that the intracellular muscle cell is hypertonic. Therefore, the muscle tissues will swell and many instances rupture if the amount of water absorbed exceeds the capacity of the cells. SEL has been implicated in reactive oxygen metabolite (ROM) production. Cells that contain larger amounts of ROM experience compromised cellular membrane integrity. Therefore animals fed SEL Se have a high probability of increased drip loss by reason of ROM production.

The serum T₃ data suggest that supplemental Se is necessary for the increased conversion of serum T₄ to T₃. This observation further suggests that the background level of Se in the basal diet was not sufficient to effectively meet the requirements of the high yielding broiler line utilized in this study. The ratios between serum T₄ and T₃ indicated that at as early as two weeks of age, the ratio between the thyroid hormones were elevated

significantly in those broilers given diets without supplemental Se as compared to those broilers given SEL or SP. Additionally, the ratios between the hormones strongly suggest that organic Se supplementation facilitated the conversion of T_4 to T_3 . This observation suggest that the extra-thyroidal conversion of T_4 to T_3 was mediated by the hepatic Se dependent type I, 5'-iodothyronine deiodinase enzyme (Edens, 2001).

The thyroid hormone data suggest that conversion of T_4 to T_3 was more efficient when SP was the supplemented Se source. Because thyroid hormones are implicated in feathering, the slightly higher levels of T_3 in SP fed birds could be linked to the improved feathering rate seen in birds given supplemental SP (Edens, 1996; Edens *et al.*, 2000).

CONCLUSIONS

These beneficial effects of SP on production performance and physiological responses in broiler chicks support the conclusion that selenomethionine may be a superior form of Se supplementation for today's poultry industry.

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Table 1. Influence of selenium source on body weight, feed conversion, mortality and feather weight of broilers.

Treatment		Body Weight (kg)	Feed Conversion (1-42 days) Feed/BW Gain	Mortality %	Feather Weight % Live BW
No Selenium	Added	2.38 ^b	1.93 ^a	2.3 ^a	6.24 ^c
0.2 ppm Selenite		2.43 ^{ab}	1.87 ^b	2.5 ^a	6.55 ^b
0.2 ppm Sel-Plex		2.45 ^a	1.84 ^{bc}	2.3 ^a	6.95 ^a
0.1 ppm Selenite		2.45 ^a	1.82 ^c	2.3 ^a	6.62 ^{ab}
0.1 ppm Sel-Plex					

^{a,b,c} Means within a column with different superscripts are significantly different ($P < 0.05$).

Table 2. Influence of selenium source on percentage yield of parts based on carcass weight.

Selenium Source	Visceral	Feet	Head	Neck	Fat Pad	Rib
No Selenium Added	13.84 ^{ab}	5.13 ^b	2.96 ^a	5.04 ^{ab}	1.47 ^a	6.35 ^a
Selenite 0.2 ppm	13.91 ^{ab}	5.33 ^{ab}	2.96 ^a	4.84 ^b	1.47 ^a	6.58 ^a
Sel-Plex 0.2 ppm	14.78 ^a	5.42 ^a	3.01 ^a	5.26 ^a	1.50 ^a	6.57 ^a
Sel-Plex, 0.1 ppm + Selenite, 0.1 ppm	13.29 ^b	5.38 ^{ab}	3.07 ^a	4.96 ^{ab}	1.46 ^a	6.69 ^a
SEM	0.35	0.10	0.06	0.11	0.02	0.17

^{a,b} Means within a column with different superscripts are significantly different ($P < 0.05$).

Table 3. Influence of selenium source on percentage yield of parts based on carcass weight.

Selenium Source		Drum	Thigh	Wing	Pectoralis Major	Pectoralis Minor	Breast Skin	Back
No Selenium Added		12.18 ^b	15.06 ^a	9.56 ^a	16.69 ^a	4.28 ^a	2.43 ^a	18.25 ^a
Selenite, 0.2 ppm		12.58 ^{ab}	15.29 ^a	9.73 ^a	16.37 ^{ab}	4.15 ^a	2.39 ^a	17.57 ^a
Sel-Plex, 0.2 ppm	0.2	12.86 ^a	15.49 ^a	9.87 ^a	15.60 ^b	4.14 ^a	2.48 ^a	18.02 ^a
Sel-Plex, 0.1 ppm + Selenite, 0.1 ppm	0.1	12.58 ^{ab}	15.44 ^a	9.70 ^a	16.12 ^{ab}	4.21 ^a	2.37 ^a	17.76 ^a
SEM		0.18	0.24	0.19	0.32	0.07	0.10	0.27

^{a,b} Means within a column with different superscripts are significantly different ($P < 0.05$).

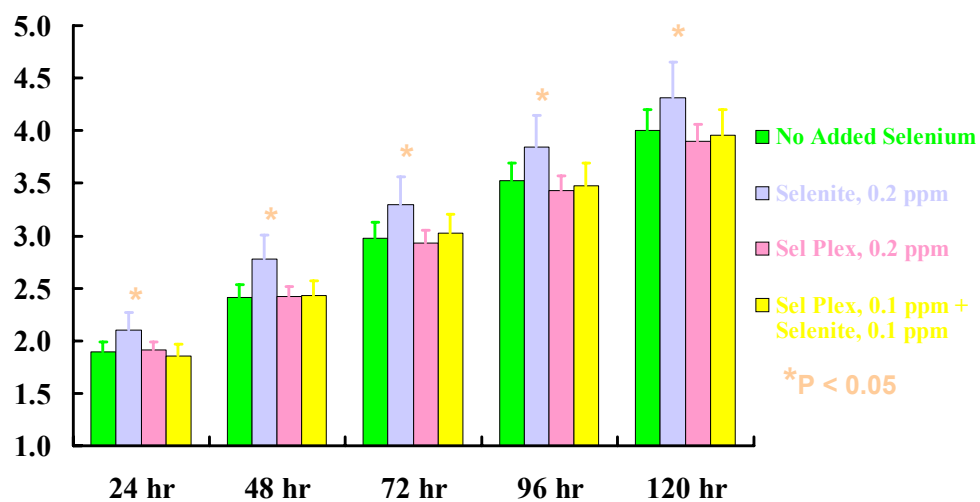


FIGURE 1. Effects of selenium source on drip loss from broiler breast meat

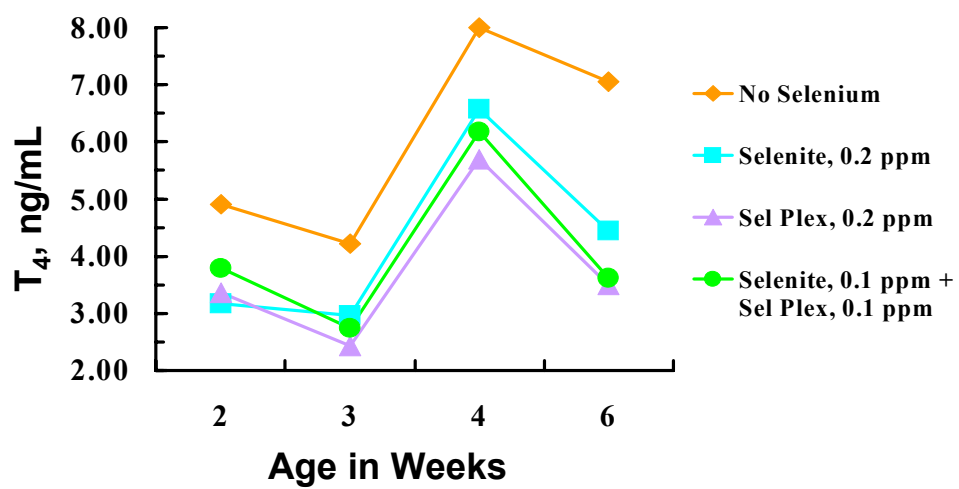


FIGURE 2. Effects of selenium source on thyroxine of broiler chickens

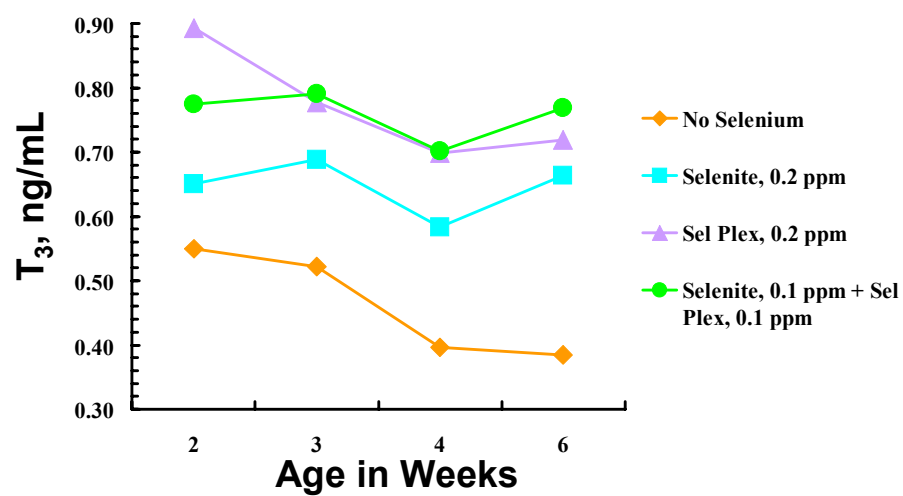


FIGURE 3. Effects of selenium source on serum triiodothyronine of broiler chickens.

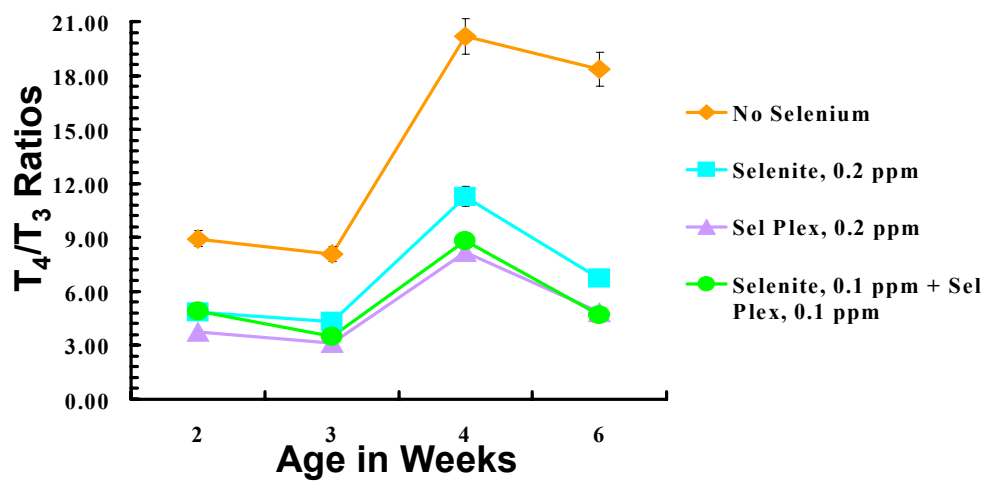


FIGURE 4. Effects of selenium source on serum T₄/T₃ ratios of broiler chickens.

Manuscript II: The Effects of Feeding Oxidized Fat and Selenium Source on Performance, Glutathione Peroxidase, and Glutathione Reductase Activity in Broiler Chickens

ABSTRACT An adequate or elevated selenium status of broilers, influenced by dietary selenium sources, improves the bird's ability to overcome the adverse effects of reactive oxygen metabolites. The objective of this study was to evaluate the effects of feeding graded levels of oxidized poultry fat on blood and hepatic glutathione peroxidase (GSH-Px), and hepatic glutathione reductase (GR) activity in broiler chickens given either, inorganic, sodium selenite, or organic selenium, enriched in the selenium yeast product, Sel-PlexTM. Nine starter diets with varying levels of oxidized fat (ca. 0, 3, or 6 meq of peroxides in poultry fat/kg of finished feed) and dietary Se sources (provided as either (1) no supplemental Se (Basal), (2) selenomethionine (SP, Sel-PlexTM; Alltech, Inc.) or (3) sodium selenite (SEL) were fed to 360 male chicks from hatch to 21 days of age using a 3 X 3 factorially-arranged completely randomized statistical design. SP and SEL were added to the basal diet to provide either 0 or 0.2 ppm of supplemental Se in the diets. Blood and hepatic samples were obtained from five birds for each treatment group at 21 days of age. Neither oxidized fat nor Se source significantly altered the activity of hepatic GR ($P > 0.05$). Blood GSH-Px was influenced significantly by both fat and Se source ($P < 0.05$), but the fat X Se source interaction was not significant ($P > 0.3$). There was a Se source effect on the hepatic GSH-Px activity ($P < 0.05$) as shown by an elevated GSH-Px activity even in the basal diet with no added oxidized fat. There was no peroxide level effect on GSH-Px in the SEL group, but GSH-Px activity in the SP group did not increase until dietary peroxide level was at 6 meq/kg. Elevated GSH-Px activity is indicative of oxidative stress, and therefore it was concluded that dietary SP supplementation resulted in better selenium status in broilers than

did SEL. Dietary inclusion of the highest level of oxidized fat was necessary to impose sufficient oxidative stress in the SP-fed birds to induce hepatic GSH-Px activity. These results indicate that the dietary Se supplied in an organic form (selenomethionine) improved Se status in broilers, leading to greater resistance to oxidative stress than when inorganic form of Se (sodium selenite) was fed.

Key Words: selenium, selenomethionine, glutathione peroxidase, glutathione reductase

INTRODUCTION

Efficient broiler production is facilitated by the feeding of well-balanced diets to highly productive lines of birds. Natural antioxidants play an important role in maintaining bird health, productivity, and reproductive characteristics. Oxidation of fats in feeds is known to produce undesirable results, such as loss of fat-soluble vitamins, degradation of xanthophyll and carotene, generation of malodors, palatability problems and even certain deficiency syndromes (Calabotta and Shermer, 1985). Products of lipid oxidation and/or destruction of the vital nutrients have been implicated as causes of steatitis in swine and cats, encephalomalacia in poultry and, symptomatically, may become manifest as exudative diathesis, muscular dystrophy, necrotic tissue in various organisms and/or poor fertility and hatchability (Ames and Swanson, 1958; Arscott et al., 1965; Bieri et al., 1961; Machlin et al., 1959; Machlin and Gordon, 1962; Rasheed et al., 1963). These are readily detectable consequences of feeding severely oxidized feed but there are other, more subtle consequences, such as reduced body weight and feed efficiency (Calabotta and Shermer, 1985). These can be difficult to detect, and their economic consequences are even more

difficult to quantify. Therefore antioxidants are added to feeds to help protect against these problems (Surai, 2002).

Many of the oxidative processes that occur in animal diets have been characterized and the mechanisms by which these processes occur have been defined (Shermer and Calabotta, 1985). Free radicals formed by unsaturated fatty acids react with oxygen to form peroxides that serve as the entry point to a multitude of reactions producing numerous byproducts and the decomposition of vital nutrients contained in the feed (Shermer and Calabotta, 1985).

An excessive production of oxygen based free radicals, especially the highly toxic hydroxyl radical (OH), leads to indiscriminate damage to macromolecules, including DNA, proteins and membrane lipids (Halliwell et al., 1992). To resist damage due to the presence of reactive oxygen metabolites, organisms have evolved non-enzymatic and enzymatic antioxidant defense mechanisms. The non-enzymatic antioxidant defense mechanisms include direct free radical scavengers, such as vitamin E, vitamin C, and glutathione (Dylock, 1995) and a number of iron chelators such as ferritin (Halliwell et al, 1992). The enzymatic defenses are provided by superoxide dismutase, glutathione peroxidase (GSH-Px), glutathione reductase (GR) and glucose-6-phosphate dehydrogenase, the limiting factor in NADPH production (Singh and Pathak, 1990). Both GSH-Px and GR are involved in the redox cycling of glutathione (Pablos et al, 1998). GSH-Px oxidizes glutathione when reducing toxic endogenous and exogenous peroxides, while GR restores the reduced glutathione status (Pablos et al, 1998).

Vitamin E is widely supplemented in poultry diets and the level of its supplementation has been increased several fold during the last few years (Surai, 1999). In

more recent years, carotenoids have been included in the antioxidant family, however their definitive role in avian nutrition awaits investigation (Surai and Speake, 1998; Surai et al., 2001; 2001a). Generally, an integrated antioxidant system has been described in avian tissues (Surai, 1999); and it has been suggested that the cell's first line of antioxidant defense is based on the activity of three enzymes; glutathione peroxidase, superoxide dismutase, and catalase. GSH-Px has received only limited attention in relation to its effects on poultry production.

Se is an essential trace element supplemented in poultry diets for maintenance of the GSH-Px cellular antioxidant system. In the chicken, Se deficiency, especially in combination with low vitamin E supply, is responsible for the development of a range of diseases including exudative diathesis (Noguchi et al., 1973; Barthlomew et al., 1998), nutritional encephalomalacia (Century and Hurwitt, 1964; Combs and Hady, 1991) and nutritional pancreatic atrophy (Thompson and Scott, 1969; 1970; Cantor et al., 1975a). It has been postulated that lipid peroxidation is important in the etiology of these diseases (Fraga et al, 1987). In this regard, progress in understanding the importance of different selenoproteins as elements of antioxidant systems could aid in explaining some of the clinical signs of the diseases. Furthermore the importance of the selenoproteins' functions in the antioxidant system need to be further investigated. The following experiment were conducted to examine the positive responses of organic Se supplementation seen in broiler chickens (Edens, 1996; Edens et al., 2000; Naylor et al., 2000; Norheim and Moksnes, 1985; Moksnes and Norheim, 1986) and to determine if the functions of the selenoproteins, GR and GSH-Px, are partially responsible for the favorable results that have been documented.

MATERIALS AND METHODS

Animal Welfare. This project was approved and conducted under the supervision of the North Carolina State University Animal Care and Use Committee which has adopted Animal Care and Use Guidelines governing all animal use in experimental procedures.

Animals and Experimental Treatments. Three hundred sixty Cobb-500 male chicks were obtained from the North Carolina Agricultural Research Service Poultry Field Research Laboratory. On the day of hatching, the chicks were individually neck-banded³ and placed in groups of ten and body weights were recorded. The chicks were randomly placed in Petersime battery brooders⁴ and feed was added to all stainless steel pens. Water in stainless steel pans and feed were available *ad libitum* to all chicks in the experiment. The nine starter diets consisted of varying levels of oxidized fat (*ca.* 0, 3, 6 meq oxidized poultry fat/kg finished feed) and dietary Se sources (provided as either (1) no supplemental Se (2) selenomethionine (SP, Selplex™; Alltech, Inc) or sodium selenite (SEL)). Unstabilized poultry fat was chosen and oxidation of fats was produced by heating them to 80 °C. Bubbling air was introduced into the reactor with stirring until the desired level of peroxide production was reached (Figure 1). Peroxide values were calculated by performing peroxide value tests (Cd 8b-90) according to the methods of the Association of Official Chemists' Society (1984). The composition of the basal diet used in this experiment is presented in Table 1. The feed was stored at -15°C until feed was fed to birds to reduce potential of further autoxidation. Body weights and feed conversions (FCR) were determined on a weekly basis for three weeks.

³ Heartland Animal Health, Fair Play, MO 65649

⁴ Petersime Equipment Company

At three weeks of age, five birds from each treatment were randomly chosen for blood and tissue sampling. Blood samples were obtained *via* cardiac puncture and collected in heparinized vials for storage for both whole blood and erythrocyte analysis. Chickens were then killed by CO₂ asphyxiation and hepatic samples (1g) were collected for enzyme analyses.

Cytosolic Protein Extraction. The dissected liver tissue was placed directly in ice-cold protein sample buffer (50 mM KPO₄, pH=7.4, 1 mM PMSF, and 5 mM EDTA). A sample (0.5-1.0 gm) from each liver was washed three times to rinse the blood from the samples and then minced in 4mL of cold protein sample buffer for protein extraction. Subsequently the samples were homogenized with a Polytron® homogenizer⁵. The homogenates were centrifuged at 6,500 X G for 30 min. Then the supernatants were removed and further centrifuged at 50,000 X G for 30 minutes. Total protein content was measured using a Protein Assay Kit⁶ with bovine serum albumin (BSA⁷) as a standard. The standard curve was linear ($r^2 \geq 0.98$) up to 80 µg/mL of BSA.

Whole Blood Glutathione Peroxidase and Erythrocyte Glutathione Reductase Analysis. Whole blood GSH-Px activity was determined in triplicate samples by spectrophotometric method modified for a plate reader⁸ based on the decrease in NADPH absorbance at A₃₄₀. The rate of decrease at A₃₄₀ is directly proportional to the GSH-Px activity (Oxis International Inc., 1997).

⁵ Heat System Ultrasonics, Plainview, NY 11803

⁶ Bio-Rad, Hercules, CA 94547

⁷ Sigma, St. Louis, MO 63178

⁸ OXIS International, Portland, OR 97217

Erythrocyte Glutathione Reductase. Erythrocyte GR was determined by a spectrophotometric method based on the measurement of the consumption of NADPH at A_{340} (Oxis International Inc., 1997).

Hepatic Glutathione Peroxidase and Glutathione Reductase Analysis. The dissected liver tissue was placed directly in ice-cold protein sample buffer (50 mM KPO_4 , pH=7.4, 1 mM PMSF, and 5 mM EDTA). A sample (0.5-1.0 gm) from each liver was washed three times to rinse the blood from the samples and then minced in 2mL of cold protein sample buffer. Subsequently the samples were homogenized with a Polytron® homogenizer². The homogenates were centrifuged for 30 minutes at 6,500 X G. Subsequently, 1 ml of the supernatant from the homogenate was further centrifuged at 50,000 X G for 30 minutes at 4°C. GSH-Px activity was determined by spectrophotometric method modified for a plate reader⁵ based on the decrease in NADPH absorbance at A_{340} . The rate of absorbance decrease at A_{340} is directly proportional to the GSH-Px activity. Hepatic GR was determined by a spectrophotometric method based on the measurement of the consumption of NADPH at A_{340} .

Statistical Analysis. Data from the experiment were subjected to statistical analysis using the General Linear Model procedures of the Statistical Analysis System (SAS, 1996). A 3 X 3 factorial arrangement of treatments was implemented with the main effects being Se supplementation and level of oxidized fat inclusion; interactions of main effects were also determined. Differences among main effect means and interactions ($P \leq 0.05$) were assessed using least squares comparisons (SAS, 1996).

RESULTS

Performance. Mean BW and FCR were not affected significantly by Se source or fat oxidation among the nine treatments. The results obtained at 7, 14, and 21 days are summarized in Table 2. The background Se level in the basal diet was apparently adequate to prevent development of any extreme, classical signs of Se deficiency during the experimental period. Se supplemented and non-supplemented birds had similar weight gains.

Glutathione Peroxidase. The effect of dietary supplementation with different Se sources and oxidized fat on GSH-Px activity in the erythrocytes is shown in Table 3. Blood GSH-Px was influenced significantly by both fat and Se source ($P < 0.05$), but the fat X Se source interaction was not significant ($P > 0.3$). The effect of dietary supplementation with Se source and oxidized fat on hepatic GSH-Px is shown in Table 4. There was a Se source effect on the hepatic GSH-Px activity ($P < 0.05$) with SEL causing an elevated GSH-Px activity even in the basal diets with no added oxidized fat. There was no peroxide level effect on GSH-Px in the SEL group. However GSH-Px activity in the SP group did not increase until dietary peroxide level was at the highest inclusion level, 6 meq/kg.

Glutathione Reductase. The effect of Se source supplementation and oxidative inclusion on GR activity in hepatic tissues is shown in Table 5. In general, GR activity in hepatic tissues of Se supplemented animals was not statistically different ($P > 0.05$) from that in tissues of non-supplemented animals, indicating that Se supplementation alone did not alter the activity of this enzyme. The enzyme activity was not affected by peroxide in either the Se-supplemented or non-supplemented group. Linear regression analysis of the data failed to indicate any significant correlation between GSH-Px and GR activities.

DISCUSSION

The effect of feeding high peroxide levels on performance is controversial. Research has found that increased peroxide levels do affect performance (Nakamura et al., 1972; Reddy and Tappel, 1974; Inoue et al, 1984; Cabel et al, 1988). When feeding elevated levels of peroxidized fat, opposing results have also been reported (Lea et al., 1964, 1966; Carpenter et al.1966; L'Estrange et al.1966, 1967;Runnels et al., 1966;Oertel and Hartfiel 1982; Jenkins and Emmons, 1984). The data presented in this report agree with the latter.

The Se-dependent glutathione peroxidase system (GSH-Px) is vital for protection against tissue peroxidation and requires the following nutrients: Se as a component of GSH-Px , the sulfur amino acids in the form of cysteine in glutathione (GSH), riboflavin in flavin adenine dinucleotide (FAD) for GR activity and niacin as a component of reduced nicotinamide adenine dinucleotide phosphate (NADPH).

The GSH-Px activity in the erythrocytes and hepatic tissue yielded some unexpected results. Certain non-supplemented Se treatments provided enzyme activities that were not statistically different from that of Se supplemented diets. These results are in agreement with previously reported data from Lawrence and Burk (1976; 1978). The hypothesis for this effect may reside in the basic theories of enzymology. When Se is sufficient in the diet, the enzyme binds substrate and could reach a maximum activity. However, when Se is deficient, there is no substrate to bind to. Additionally, when there is substrate added, an immediate increase in enzyme activity may occur.

The higher activity of hepatic GSH-Px in Se-supplemented animals than in non-supplemented animals is in accordance with various literature reports (Chow and Tappel, 1974; Naguchi et al., 1973; Omaye and Tappel, 1974a; 1974b). Previous experiments with

animals have detected significant differences among various Se compounds in their capacity to meet Se requirement of the animals. Also, there are differences reported for the same compounds in their effectiveness to protect against many of the manifestations of Se deficiency. The conflicts in these reports are likely due to the confounding effect of the background Se levels in the experimental diets. If the background levels of Se are high, the effects of Se supplementation can be masked. Comparative efficiency of dietary Se compounds, especially sodium selenite and selenomethionine, in raising GSH-Px activity is also the subject of much controversy. A roughly equal availability of Se from selenomethionine and sodium selenite in chicks (Omaye and Tappel, 1974a) and rats (Pierce and Tappel, 1977) has been documented, but Cantor et al. (1975b) revealed a higher utilization of sodium selenite in chicks as compared with that from selenomethionine. The difference in the basal diets used by these two groups of investigators, in particular the presence and absence of vitamin E supplement as was pointed out by Pierce and Tappel (1977), may be a possible factor modifying the effectiveness of the two compounds.

Wang and Spallholz (1980) reported that selenomethionine restored rat liver GSH-Px activity much more slowly than sodium selenite or high Se yeast in Se-depleted rats. Yet, Schwartz and Foltz (1958) reported that sodium selenite and DL-selenomethionine had similar factor 3, now referred to as GSH-Px, activity in rats. Sunde and Hoekstra (1980) have shown with perfused rat liver that sodium selenite and sodium selenide are metabolically closer than selenocysteine to the immediate Se precursor used for GSH-Px synthesis. Because GSH-Px activity for the hepatic tissue was not increased when selenomethionine was fed as the supplemental Se source rather than sodium selenite, it seems that selenomethionine must be incorporated into some other protein in the tissues. In fact,

cases where supplementation of selenomethionine is at a high level in feeds, it has been demonstrated that 40 to 50% of total body Se as selenomethionine can be found in muscle (Daniels, 1996). Selenocysteine is the pivotal amino acid in the synthesis of Se-dependent cytosolic GSH-PX (Rotruck et al, 1973), but only about 30% of the body's Se is incorporated into cytosolic GSH-Px. About 70% of the body's Se is incorporated into 30 to 100 other selenoproteins in mammals (Burk and Hill, 1993).

The stored form of organic Se is in a non-functional state and therefore not used immediately for formation of biologically functional selenoproteins (Mahan, 1994; 1995). Since this non-functional Se is better retained than inorganic Se, its conversion to selenide before conversion to selenocysteine and other functional selenoproteins appears to be more efficient. As a consequence of the large pool of stored Se in protein, in times of oxidative stress, body protein can be degraded rapidly providing more than adequate concentrations of organic Se that can be used for synthesis of specific selenoproteins such as Se-dependent GSH-Px enzymes.

Because elevated GSH-Px is also indicative of oxidative stress (Omaye and Tappel, 1974b; Hull and Scott, 1976), the dietary SP supplementation resulted in better Se status in broilers than in birds fed sodium selenite. Only dietary inclusion of the highest level of oxidized fat was necessary to impose sufficient oxidative stress in the SP fed birds to cause a rise in hepatic GSH-Px activity.

Since the complete GSH-Px system responds to conditions that induce lipid peroxidation (Chow and Tappel, 1972; Chow et al., 1973), GR activity was also measured. In general, GR activity in hepatic tissues of Se supplemented animals was not statistically different ($P>0.05$) from that in tissues of non-supplemented animals, indicating that Se

supplementation alone did not alter the activity of this enzyme. The enzyme activity was not affected by peroxide in either the Se-supplemented or non-supplemented group, which was indicative that GR activity in the tissues analyzed, was adequate to provide reduced glutathione for reduction of additional peroxides. The GR activity in tissues from the peroxide-fed groups was also essentially the same. The findings that Se source fed did not affect GR activities in tissues agrees with other reports (Hull and Scott, 1976; Parsons et al., 1985). Linear regression analysis of the data failed to indicate any significant correlation between GSH-Px and GR activities which was in agreement with other published studies (Godin and Garnett, 1992; Perez-Campo et al., 1993; Venditti et al., 1999). These findings suggest that tissue levels of antioxidant enzymes may be independently regulated.

Although we incorporated poultry fat with no additional stabilizers in the diets, there are reports of ethoxyquin carryover in poultry fats (P. R. Ferket, 2002, NCSU, Raleigh, NC, personal communication). If there is carryover of the ethoxyquin stabilizer in the fat, less antioxidant is required for optimum performance. Furthermore, the addition of this ethoxyquin in the diet could confound the effect of oxidized fat on the performance data as well as the antioxidant enzymes that were measured.

CONCLUSION

The results from this study indicate that the dietary Se supplied in an organic form (selenomethionine) may improve Se status in broilers. This leads to greater resistance to oxidative stress than when an inorganic form of Se (sodium selenite) is fed.

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TABLE 1. Diet Composition

Ingredient	Amount (%)
Ground Yellow Corn	53.71
Soybean Meal (48% Protein)	36.92
Poultry Fat	5.38
Dicalcium Phosphate	1.88
Ground Limestone	1.16
Salt	.51
Choline Chloride (60%)	.20
D,L-Methionine	.13
Mineral Premix ¹	.05
Vitamin Premix ²	.05
Total	100

¹Supplies the following per kilogram of feed: zinc 120 mg ; manganese 120 mg; iron 80 mg; copper 10 mg; iodine 2.5 mg; cobalt 1 mg

²Supplies the following per kilogram of feed: vitamin A 6,600 IU; vitamin D₃ 2000 ICU; vitamin E 33 IU; vitamin B₁₂ 19.8 µg; riboflavin 6.6 mg; niacin 55 mg; d-Pantothenate 11 mg; menadione 2 mg; thiamine 2.0mg; pyridoxine 4 mg; biotin 126 µg; folic acid 1.1 mg

TABLE 2. Influence of selenium source and oxidized fat on broiler performance¹.

		Days of age			
		Body weight			
Selenium	Oxidized fat	7	14	21	FCR
0	0	0.159	0.44	0.824	1.54
	3	0.163	0.448	0.849	1.43
	6	0.176	0.471	0.896	1.47
0.2 ppm Selenite	0	0.161	0.452	0.919	1.44
	3	0.413	0.414	0.826	1.55
	6	0.165	0.462	0.887	1.48
0.2ppm Sel-Plex	0	0.153	0.427	0.838	1.61
	3	0.166	0.449	0.845	1.54
	6	0.152	0.427	0.81	1.44
Source of variation		Significance			
Selenium source		NS	NS	NS	NS
Peroxide level		NS	NS	NS	NS
Se x P		NS	NS	NS	NS
SEM (35) ²		9.6	17.5	35.1	0.06

¹Means represent average of 4 pens/treatment with *ca.* 10 birds per pen

²SEM= Standard error of the mean with 35 degrees of freedom

NS= P>0.05

TABLE 3. Influence of selenium source and oxidized fat on erythrocyte GSH-Px. Data are expressed as nmol/mg Hb¹

Selenium source	Oxidized fat (meg peroxide/kg feed)	Erythrocyte GSH-Px
0	0	269
	3	1600
	6	764
0.2 ppm Selenite	0	307
	3	1738
	6	1005
0.2ppm Sel-Plex	0	439
	3	2318
	6	1009
Source of variation		Significance
Selenium source		*
Peroxide level		***
Se X P		NS
SEM (35) ²		160.5

¹Means represent average of 5 birds/treatment

²SEM= Standard error of the mean with 35 degrees of freedom

*P<0.05

**P<0.01

***P<0.005

NS=P>0.05

TABLE 4. Influence of selenium source and oxidized fat on hepatic GSH-Px. Data are expressed as nmol/mg of total protein¹.

Selenium source	Oxidized fat (meg peroxide/kg feed)	Hepatic GSH-Px
0	0	292
	3	526
	6	202
0.2 ppm Selenite	0	309
	3	543
	6	296
0.2ppm Sel-Plex	0	328
	3	600
	6	629
Source of variation		Significance
Selenium source		*
Peroxide level		**
Se X PV		NS
SEM (29) ²		88.2

¹Means represent average of 5 birds/treatment

²SEM= Standard error of the mean with 29 degrees of freedom

*P<0.05

**P<0.01

***P<0.005

NS=P>0.05

TABLE 5. Influence of selenium source and oxidized on hepatic GR. Data are expressed as mU/mg of total protein¹.

Selenium source	Oxidized fat (meq peroxide/kg feed)	Hepatic GR
0	0	214
	3	322
	6	280
0.2 ppm Selenite	0	232
	3	225
	6	230
0.2ppm Sel-Plex	0	229
	3	262
	6	283
Source of variation		Significance
Selenium source		NS
Peroxide level		NS
Se X PV		NS
SEM (36) ²		48.9

¹Means represent average of 5 birds/treatment

²SEM= Standard error of the mean with 36 degrees of freedom

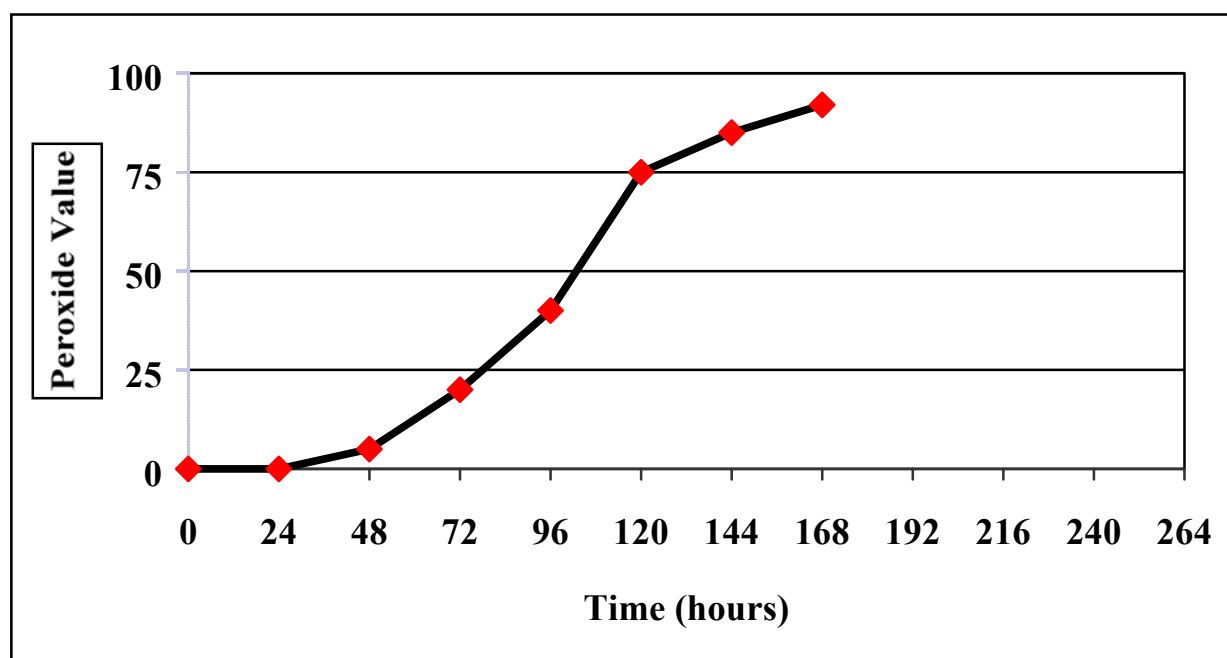
P<0.05

**P<0.01

***P<0.005

NS=P>0.05

FIGURE 1. Oxidation of poultry fat incorporated in experimental diets.



General Summary

In the last few years, a great deal has been learned about the supplementation of organic Se to poultry diets. There is a developing database supporting the hypothesis that selenomethionine plays important roles in poultry diets.

Se is a unique trace mineral because it is the only trace mineral with a special mRNA (Allan et al, 1999) and a special tRNA (Commans and Bock, 1999) that are required for its incorporation into the amino acid, selenocysteine. The natural form of Se in plants and grains is selenomethionine, and its conversion to selenocysteine for incorporation into Se-dependent proteins, such as glutathione peroxidase and hepatic iodothyronine deiodinase appears to be more efficient.

These studies demonstrate the positive influence of organic Se supplementation on the performance of broiler chickens when reared to market age. The body weights at 42 days was increased in selenomethionine-fed broilers as compared to those given no supplemental Se or birds fed sodium selenite. Even though background levels of Se were sufficient to sustain good performance by the broilers involved in this study, additional Se appeared to be necessary to optimize growth. Feed conversions were improved by both Se sources when compared to the FCR for broilers given no supplemental Se in their diets. These data suggest that an additional requirement for Se may be necessary for the modern fast growing, high yielding broiler chicken, and selenomethionine in selenized yeast appeared to be better than sodium selenite in meeting this additional requirement.

In these studies we show that selenomethionine in selenized yeast was more efficient than sodium selenite in reducing the frequency of pale, soft, exudative (PSE) breast meat of our broilers. The lower level of selenomethionine was equivalent to or better than the higher

level of dietary sodium selenite in reducing the frequency of PSE meat. Our data clearly demonstrate that there may be an oxidative stress that contributes to the development of PSE meat.

The studies herein show that on a percentage of carcass weight, yields of viscera, feet, and neck were increased with the feeding of selenomethionine in Se yeast as compared to sodium selenite. Yields of leg, as a percentage of carcass weight, were increased with selenomethionine feeding, but yields of *pectoralis major* were slightly decreased with selenomethionine feeding as compared to sodium selenite.

Drip loss from broiler breast meat was evaluated in our studies and the data show that it was neither the lack of supplemental Se in the basal diet nor the supplementation of selenized yeast that decreased drip loss, but it was the presence of sodium selenite that induced drip loss to a higher percentage than did selenized yeast or the organic Se in the basal diet.

We also investigated the status of serum thyroxine levels in birds that had been given diets with no supplemental Se as compared to sodium selenite and to Se yeast supplemented diets. The serum triiodothyronine levels were lower in broilers that had been given no supplemental Se in their diets as compared to broilers that had been supplemented with selenite or selenomethionine in their diets. The ratios between the two serum thyroid hormones were significantly higher than broilers given diets without supplemental Se as compared to those broilers fed sodium selenite or selenomethionine in selenized yeast.

The thyroid hormone data in our study was a minor issue, but it appeared that selenomethionine feeding might have stimulated the conversion of thyroxine to triiodothyronine by hepatic iodothyronine deiodinase, which is a Se dependent enzyme.

Additional work was warranted to further explain the positive performance associated with supplementation of selenomethionine in broiler diets. In a second study, the antioxidant enzymes, glutathione peroxidase and glutathione reductase, were measured in broilers supplemented selenomethionine and sodium selenite and oxidatively challenged with peroxidized fat supplementation.

Blood GSH-Px was influenced significantly by both fat and Se source, but the fat X Se source interaction was not significant ($P>0.3$). There was a Se source effect on the hepatic GSH-Px activity with sodium selenite causing an elevated GSH-Px activity even in the basal diets with no added oxidized fat. There was no peroxide level effect on GSH-Px in the sodium selenite group, but GSH-Px activity in the Se yeast group did not increase until dietary peroxide level was at the highest inclusion level, 6 meq/kg.

In general, GR activity in hepatic tissues of Se supplemented animals was not statistically different from that in tissues of non-supplemented animals, which indicated that Se supplementation alone did not alter the level of this enzyme. The enzyme level was not affected by peroxide in either the Se-supplemented or non-supplemented group. These observations were indicative that GR levels in the analyzed tissues were adequate to recycle reduced glutathione for reduction of additional peroxides. The GR activity in tissues from the peroxide-fed groups was also essentially the same.

Although we incorporated poultry fat with no additional stabilizers in the diets, there are reports of ethoxyquin carryover in poultry fats (P. R. Ferket, 2002, NCSU, Raleigh, NC, personal communication). If there is carryover of the ethoxyquin stabilizer in the fat, less antioxidant is required for optimum performance. Furthermore, the addition of this

ethoxyquin in the fat source could confound the effect of oxidized fat on the performance data as well as the antioxidant enzymes that were measured.

The supplementation of organic Se as selenized yeast was shown to impart an anti-stress condition in broilers subjected to oxidative stress. In cells that may contain large amounts of ROM, cell membranes can rupture because cellular membrane integrity is compromised. This is a high probability in animals that are fed diets supplemented with sodium selenite Se because sodium selenite Se is known to be responsible for ROM production. The improved stress resistance in the selenized yeast-fed broilers was due to more efficient scavenging of ROM than in birds that were not given the Se yeast supplement.

Therefore it was concluded that Se yeast may provide a superior form of Se for poultry. The beneficial effects of Se yeast on production performance and the antioxidant enzyme profile of broiler chickens appeared to support this conclusion.