Review Paper

All Species

A role for Sel-Plex™, a source of organic selenium in selenised yeast cell wall protein, as a factor that influences meat stability

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Summary

Selenium is an important mineral required in the antioxidant system in animals, which is involved with oxidative stability in tissues, particularly membranes, and is involved in various aspects of meat quality and stability on the shelf, due to its protective properties on lipids, preventing rancidity. Se can be supplied in an inorganic or chemically organic form, and it is well known that the latter has beneficial properties and improved functionality in physiological systems compared to the former. Research has shown that organic Se is associated with increased tenderness and the prevention of certain problems in pale exudative meat, discoloration and off-flavours and odours in meat, although this depends on other components of the antioxidant system, such as vitamin E, being present as well. The change in prominence of glutathione peroxidase forms in their interaction with vitamin E in cell membranes is also noted. The following review (the third in a series) details the research that has been conducted into the role of Se in meat stability and related factors, with specific focus on organic forms of Se, namely the commercial product Sel-Plex™ (Alltech Inc, Nicholasville, KY, USA), which is derived from yeast and in which selenium replaces sulphur in methionine forming selenomethionine in yeast protein.

Keywords: selenium; meat stability; antioxidants; selenoenzymes; glutathione peroxidases

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Introduction

The importance of Se in nutrition and incorporation into tissues including meat has been covered in recent reviews (Edens and Sefton, 2016a, b). In this final review, it is important to address the issue of selenium forms that have an influence on meat stability. Additionally, it is important to attempt to explain how selenoproteins such as the glutathione peroxidases (GSHpx) play an integral role in maintenance of meat stability, and why organic selenium has a greater positive influence on meat stability than inorganic selenium in selenite or selenate forms.

Selenium influence on oxidative status of meat

A recent review by Estévez (2015) on the supplementation of organic forms of selenium to feed espoused the concept that selenium, especially organic selenium, is required for the prevention of lipid and protein oxidation that decreases broiler growth via promotion of oxidative stress, which then leads to decreased meat quality. Following early studies showing that organic selenium yeast (Sel-Plex™) supplementation to broiler diets were implicated in less drip loss from refrigerated breast meat (Edens, 1996; Edens et al., 1996; Edens et al., 1998),
further interest for the use of Sel-Plex™ to improve meat stability by controlling membrane damage and reducing rancidity, has resulted in a large body of published research. The database on trials on poultry meat quality can be reviewed in various scientific papers (Downs et al., 2000; Naylor et al., 2000; Hess et al., 2003; Choc et al., 2004; Upton et al., 2008; Perić et al., 2009; Puvača and Stanacev, 2011). The use of organic selenium yeast to improve meat quality and stability includes not only poultry, but pork (Mahan et al., 1999), veal (Skrivanova et al., 2007), beef (Juniper et al., 2008; Cozzi et al., 2011), lamb (Vignola et al., 2009), goat (Sethy et al., 2014), as well as salmon and other fish (de Lyons, 1998). Organically bound selenium in meat is considered an important issue in the maintenance of beef and pork meat stability due to its support of glutathione peroxidase enzyme activity (Daun et al., 2001), and it appears that organically bound selenium in poultry meat is similarly important (Daun et al., 2004).

Important factors that influence consumer acceptance of poultry and red meat products include a large battery of organoleptic-based perceptions of meat quality, involving smell as an assessment of staleness versus freshness, texture (as dryness or moisture content), and textural qualities, which provides information concerning the firmness in both white and red meats. When the issue of meat quality is discussed, it involves multiple factors that influence the perceived tenderness/toughness, juiciness/moisture content, firmness/moisture, protein content and functionality, appearance/colour, apparent hydration and the economic value of meat (Northcutt et al., 1994). Factors that affect the quality of poultry and red meats are dependent upon the source animal’s nutrition during the growing period, severity of stressors experienced before slaughter, method of stunning, efficiency of exsanguination, processing techniques, post-mortem cooling, fresh versus frozen manipulation and even biochemical properties of the processed meats (Lyon and Lyon, 1993; Liu et al., 2003).

Oxidised lipids are a major issue for shelf life and stability in meat. Surai (2002) demonstrated that oxidative stress is due to an imbalance between the production of reactive oxygen and nitrogen species and the animal’s defence mechanisms. This may be caused by dietary intake of oxidised lipids and polyunsaturated fatty acids or by lack of intake of nutrients that support the body’s many antioxidant systems. To improve meat, producers need to combat pre- and post-slaughter oxidative stress effects on quality and stability. In the early 2000’s, meat producers had already begun to use dietary supplements to provide higher levels of vitamin E or ascorbic acid, which are known to have powerful antioxidant properties, and/or pre-slaughter withdrawal of pro-oxidant transition trace minerals. Due to the relationship between non-haem iron and copper in their pro-oxidant effects on fresh and cooked meat, many producers have deliberately removed these minerals from finisher diets to improve oxidative stability in the final meat. However, contrary to this, Yang et al. (2011) reported that dietary iron, copper, zinc, and manganese generally improved meat quality. Transition metals may increase the shear force for breast meat and had variable non-significant effects on thigh muscle shear force, implying a negative effect on tenderness, although Yang et al. (2011) made no mention of this on selenium status and oxidative status of the chicken muscles. Nevertheless, Shadhidi and Hong (1991) reported that Fe⁺², Fe⁺³, Cu⁺¹, and Cu⁺² showed pro-oxidant activity in cooked pork stored at 4 °C for 21 days and that Fe⁺² and Cu⁺¹ had greater oxidative potential than Fe⁺³ and Cu⁺². Ruiz et al. (2000) showed that removal of iron and copper from finishing diets was related to less oxidative activity in cooked broiler leg meat. The improvement in antioxidant status due to removal of iron and copper is probably due to lower content of these mineral in the meat. It is of interest that the remaining pro-oxidant iron and copper in muscle and meat retains pro-oxidant potentials. Bekhit et al. (2013) reported that these transition metals are bound to protein having limited reactive ability. After an animal has been slaughtered and the flesh matures into meat during hanging, refrigeration and during display on the grocer’s meat counters, biochemical alterations, such as pH shifts, protein and fat denaturation, and oxygen availability, which contribute to oxidative activity, can free those metals from the protein allowing them to become reactive to decrease meat quality as they generate free radicals. It is at this point that interceptive reactions from the activity of selenium-dependent antioxidant enzymes, glutathione, and other antioxidant systems can reduce newly generated free radicals.

**Muscle selenoaminoacid accumulation/retention and glutathione peroxidase activity**

Studies have shown that the feeding of selenomethionine to various animal species, including birds, mammals and fish, increased tissue and muscle Se concentration as compared with animals fed either sodium selenite or selenocysteine (Latshaw, 1975; Latshaw and Osman, 1975;
Norheim and Moksnes, 1985; Moksnes and Norheim, 1986; Deagan et al., 1987; Suomi and Alaviuhkola, 1992; Mahan, 1995; Mahan and Parrett, 1996; Payne and Southern, 2005a; Juniper et al., 2009; Wang et al., 2011a; Stewart et al., 2012; Song et al., 2015).

There are differences in storage capacity among species, which can have a depressive effect on meat quality, especially when the animals are fed sub-optimal Se levels. This can be observed in the activities of the Se-dependant antioxidant enzyme GSHpx. Daun et al. (2004) noted that oxidative meat (thigh) in poultry species had greater GSHpx activity than glycolytic meat (breast), and that duck meat had GSHpx activity fivefold higher than chicken, turkey and ostrich meats. Daun and Åkesson (2004) reported a significant positive correlation between GSHpx activity and soluble selenium content in pork and beef muscle.

A problem with inorganic selenium

Reviews of the distinct differences between inorganic selenium and selenate and the organic forms of Se derived from yeast have been completed recently (Edens and Sefton, 2016a, b). In alkaline solution, selenite selenium oxidises slowly to form the selenate ion, which is further reduced to the selenite +4 state with the presence of hydrochloric acid (IPCS, 1987), which occurs in the proventriculus of poultry and the monogastric stomach of mammals. This unstable chemical property of selenite selenium allows it to react with reducing substances such as vitamin C (Ip, 1986; Gonzalez, 1990), ferric ions (Rai et al., 1995; Edens and Sefton, 2016a), causing oxidation of the reducing agents to a non-active form, which become relatively unavailable to animals (Leeson and Summers, 1997). Inorganic Se sources have higher toxicity due to their oxidising properties, which is why many countries limit the amount of Se in the diet to 0.3 mg/kg of diet. Sodium selenite has a documented pro-oxidant property that can oxidise many substances making them non-functional (Hafeman et al., 1974; Csallany and Menken, 1986; Spallholz, 1997; Terada et al., 1999; Schrauzer, 2000).

Vitamin C is used extensively as an antioxidant and anti-stress agent, especially during times of high temperature exposure (Kolb, 1984; Mahmoud and Edens, 2003). Heat is a strong stimulus that induces oxidative stress which can damage tissues and skeletal muscles in broiler chickens, affecting meat quality (Mahmoud and Edens, 2003; Azad et al., 2010). Combating this requires greater antioxidant activity, and organic selenium as selenium yeast in Sel-Plex™ is more effective than sodium selenite in antioxidant activity via GSHpx levels (Mahmoud and Edens, 2003). In a reactive state, the pro-oxidant property of inorganic selenium is capable of oxidising many nutrients in the feed and can attack such nutrients as vitamins, fats, and even proteins. Oxidised products in feed are known to have significant negative effects on meat quality (Igene and Pearson, 1979; Asghar et al., 1989; Buckley et al., 1989; Sheehy et al., 1993b).

Since 1974, many studies have been conducted to ascertain the various roles played by selenium in the nutrition and biochemistry of poultry and livestock. In addition to the study of sodium selenite, which was chosen as the primary selenium form for animal feeds, a second branch of research has developed for the elucidation of the importance of selenomethionine as a free selenoaminoacid or in selenium yeast as an alternative to sodium selenite as a source of dietary selenium (Schruazer, 2000).

Functions of organic and inorganic selenium as antioxidants

The use of dietary selenium as an element necessary to support the glutathione (glutamylcysteinylglycine)-glutathione peroxidase (GSH) antioxidant system has greatly influenced animal nutrition. A recent meta-analysis (Bermingham et al., 2014) demonstrated that dietary selenomethionine, and selenium-yeast in particular, can increase GSHpx in selenium-enriched meat more efficiently than selenite selenium. Retention of organic selenium is longer in poultry given organic selenium than in those given sodium selenite. Under long-term depletion of selenium due to consumption of a deficient diet, increased GSHpx activity can be maintained in all tissues, including muscle, of broilers (Zhang et al., 2014) and layers (Moksnes and Norheim, 1986) which were previously fed organic selenium.

The role played by selenium-dependent GSHpx in maintaining a cellular environment favouring a reduced state is well documented. However, the relationship between GSHpx activity and GSH is often relegated to a secondary level of importance. Nevertheless, GSH is the most abundant of all of the non-protein thiols found in cells (Dickenson and Forman, 2002) and promotes the general antioxidant status within cells acting as a co-factor for all GSHpx activities (Sies, 1999; Arteel and Sies, 2001). It functions directly in the neutralisation of free radicals and serves in the maintenance of the reduced forms of the antioxidant vitamin C (Hughes,
1964) and vitamin E (Scholz et al., 1989), which is important for meat quality. Other functions in which GSH is used include metabolic and biochemical reactions such as DNA synthesis and repair, gene expression, protein synthesis, prostaglandin synthesis, amino acid transport, and enzyme activation (Sies, 1999; Arteel and Sies, 2001; Dickenson and Forman, 2002; Pomella et al., 2003). The indirect antioxidant activity of GSH in combination with GSHpx involves its use as a substrate by GSHpx in the reduction of hydroperoxides and lipid peroxides (Cohen and Hochstein, 1963). In addition to the role played by GSH as an antioxidant against reactive oxygen radicals, it interacts with peroxynitrite to reduce it to nitrite in an interaction with GSHpx (Radi et al., 1991; Sies et al., 1997). Peroxynitrite is a potent RNS radical that can cause oxidation of membrane lipids, which can affect the oxidative stability of lipids, flavour, colour and functional properties of meat (Brannan et al., 2001). The interaction between GSH and peroxynitrite is a major link in the role played by GSH in the maintenance of meat quality.

The tissue concentration of GSH is altered by its redox status in the cell and its organelles (Lu, 2013; Aoyama and Nakaki, 2015). As an antioxidant, GSH interacts with GSHpx in catalysed reactions in which hydroperoxides and lipid peroxides are reduced while GSH is oxidised to glutathione disulphide (GSSG). An important observation made by Mahmoud and Edens (2003) was that in Sel-Plex™-fed broilers subjected to a heat stressor (an inducer of oxidative stress), the reduction of GSSG to GSH was increased to meet the demands associated with the increased oxidative stress. Liver GSH and GSSG were in greater concentrations, respectively, than their concentrations in selenite and control fed broiler chickens (Mahmoud and Edens, 2003). This demonstrated that the redox balance in Sel-Plex™-fed broilers was improved compared to selenite and control fed broilers experiencing oxidative stress.

The primary source of GSH is the liver, which secretes large amounts into plasma, where it is used to maintain inter-organ GSH homeostasis (Bartoli and Sies, 1978; Ookhtens and Kaplowitz, 1998). This is an important event that has an impact on meat quality as influenced by GSH interaction with GSHpx in the control of oxidative reactions in pre-slaughter muscles and the continued oxidative and nitrosative stress in the conversion of muscle to meat. Surai (2002) suggested that the rancid condition of processed meats is initiated long before the food animal is slaughtered and processed.

The phospholipid hydroperoxide glutathione peroxidase (GSHpx-4) activity that inhibits lipid peroxidation in membranes requires the presence of both GSH and vitamin E in order for the enzyme to inhibit the rate of the initiation reactions (Ursini et al., 1986; Ursini and Bindoli, 1987; Maiorino et al., 1989 and 1991; Nomura et al., 2000). In plasma membranes, the GSHpx-4 interaction with GSH only accounts for partial inhibition of lipid peroxidation, but the presence of vitamin E in the membrane allows for nearly complete inhibition of lipid oxidation (Ursini and Bindoli, 1987). By preventing the free radical generation from lipid hydroperoxides, GSHpx-4 activity can reduce the vitamin E requirement necessary to inhibit lipid peroxidation (Ursini and Bindoli, 1987). Thus, this interaction of GSH, GSHpx-4 and vitamin E in the cell membranes is crucial in the maintenance of meat quality associated with lipid oxidation, odour and colour. The antioxidant process in cells is vital because it is an ongoing process in the living muscles and has impact in the process of conversion of muscle to meat, which becomes the basis for the relationship between selenium and vitamin E in maintenance of meat quality.

The antioxidant selenoenzyme GSHpx-4 is unique in that it has a primary residence in cell membranes where it, along with GSH, targets peroxidised phospholipids (Ursini et al., 1982 and 1985). GSHpx-4 is special in that it alone can reduce hydroperoxides in lipoproteins and other complex lipids associated with cholesterol, cholesteryl esters and phospholipids in conjunction with α-tocopherol (Maiorino et al., 1991; Liang et al., 2007). The presence of GSHpx-4 in cell membranes is very important since it reduces lipid hydroperoxides and provides protection for the integrity and stability of the cell plasma and subcellular membranes (Maiorino et al., 1991), which is important in oxidative stability in both pre-slaughter muscle and post-slaughter meat. As a selenoprotein, GSHpx-4 is sensitive to tissue selenium status. Zoidis et al. (2010) fed Sel-Plex™ (0, 0.15, 0.3, or 3.0 mg selenium/kg diet) to broiler chickens and quantified the selenium concentration in blood and liver. Increasing levels of organic selenium caused a progressive parallel increase in selenium in both blood and liver, but unlike GSHpx-1, which can be elevated in parallel with dietary selenium forms, in chickens GSHpx-4 will down regulate its expression when dietary selenium exceeds 0.15 ppm (Zoidis et al., 2010).

It has been hypothesised that poultry meat quality is improved with the use of diet-supplied antioxidants (Delles et al., 2014 and 2015). Delles et al. (2014) noted
that breast meat from broilers had significantly higher GSHpx activity and selenium content after antioxidant supplementation. They observed that high levels of oxidised fat in the diet caused decreased GSHpx activity, which was corrected by antioxidant supplements. Fellenberg and Speisky (2006) noted that feed-grade antioxidants generally improved systemic oxidative stability in broilers and that oxidative stability, related to oxidation of polyunsaturated fatty acids (PUFA), extended into the post-mortem conversion of muscle to meat. The Fellenberg and Speisky (2006) review focused on the antioxidant functions of α-tocopherol forms, β-carotene, butylated hydroxyanisole (BHA) and dibutylhydroxytoluene (BHT), tea catechins, ethyoxyquin, and the plant extracts of rosemary, sage, green tea powder, and dried tomato pulp. Each of the aforementioned antioxidants has a different mechanism of action, but the ultimate activity is to remove free radical initiators of oxidative stress and the free radical propagators of oxidative stress in muscle tissue before and after the animal has been slaughtered.

Upton et al. (2009) noted that both sodium selenite and organic selenium yeast as Sel-Plex™ increased GSHpx activity in the red blood cells and liver of broilers fed oxidised fat. However, in sodium selenite fed broilers, hepatic GSHpx was decreased by highly peroxidised dietary fat, while in broilers fed Sel-Plex™, the GSHpx activity was maintained at an elevated activity. Brigelius-Flohé (1999) wrote that GSHpx functioned in meat as a major selenium-associated antioxidant enzyme that contributes to the defence against oxidation, and Daun et al. (2004) suggested a strong relationship between muscle GSHpx activity and meat quality. In each of aforementioned trials, it is highly likely that the GSHpx-4 plus GSHpx-1 activities made a significant contribution to the antioxidant activity in the liver, muscle, and in the meat thereby rendering a positive influence on meat quality.

Biological membranes are targets for reactive oxygen (ROS) and nitrogen species (RNS) that often interact to produce peroxynitrite, which then induces lipid peroxidation of membrane phospholipid unsaturated fatty acids causing alterations in the membrane physical properties such as changes in membrane fluidity causing alterations on cell permeability and enzyme activities (Horan et al., 1994; Ahsan et al., 2003; de Lima et al., 2004). RNS are produced in animals starting with the reaction of nitric oxide (NO) with superoxide (O$_2^−$) to form peroxynitrite (ONOO$^−$; Blough and Zafririou, 1985), which are considered the most damaging of all the free oxygen/nitrogen radicals (Roussyn et al., 1996). Roussyn et al. (1996) reported that peroxynitrite caused single strand breaks in DNA, which can be blocked (75%) by selenomethionine. Briviba et al. (1996) reported that sodium selenite had no protective effects against oxidation and nitration reactions of peroxynitrite differing from the protective properties of selenomethionine. Walter and Roy (1971) reported that selenomethionine had GSHpx properties which allowed it to act in an antioxidant manner. Sies et al. (1997; 1998) further noted that in addition to selenomethionine, selenium-containing enzymes catalyse peroxynitrite reduction and that GSHpx-1 played a dual role in its ability to act as a peroxynitrite reductase. Later, Sies and Arteel (2000) reported that selenoaminoacid residues in proteins had the potential for increased moisture loss from the meat. Thus, Sel-Plex™, which contains selenomethionine, is known to improved antioxidative status of broilers via their increased selenium content in muscle and via increased activity of enzymes such as GSHpx-1 and GSHpx-4. Through implication, it can be concluded that organic selenium as selenomethionine might have more positive effects on oxidative stability and extended shelf life of fresh meat than would sodium selenite.

**Consumer issues regarding meat quality and rancidity**

Consumer assessment of meat quality is based on visual appearance, texture, firmness and smell. The odour of rancid meat is a very potent sensation that immediately results in rejection of the meat or meat product (Morrissey et al., 1998). The conditions that promote the rancid condition of processed meats is initiated long before the food animal is slaughtered and processed and is influenced by the redox state of muscle that is carried forward to the development of meat from those muscles (Surai, 2002).

In food animals, there are extensive antioxidative processes that function to control production and detoxification of ROS and RNS (Surai, 2002). Oxidative and nitrosative stressors have significant impact on the stability of tissue lipids and polyunsaturated fatty acids in both living animals and in post-slaughter meat quality and underlying the negative influence on meat quality is the potential for increased moisture loss from the meat.
Oxidative processes begun in the pre-slaughter animal continue in post-slaughter muscle as it converts to meat in both refrigerated (DeVore et al., 1983; Ryu et al., 2005 and 2006; Chen et al. 2011) and frozen states (Combs and Regenstein, 1980; Abdel-Kader, 1996).

In beef cattle, Cozzi et al. (2011) found improved body selenium and antioxidant status, which were related to improved meat quality characteristics in bulls fed selenium yeast while held in pens. Cozzi’s basal diets contained very little selenium (0.04 to 0.06 mg/kg diet). Juniper et al. (2008) found improved selenium and antioxidant status in beef cattle fed selenium yeast, but did not find any difference between inorganic and organic selenium feeding on meat oxidative stability. A major difference in the basal diet provided by Juniper was that the selenium concentration was greater (0.16 mg/kg diet) than that in Cozzi’s diet. The basal diet selenium in both studies was derived from plant-based feed ingredients, which contain selenomethionine and some selenocysteine (Burk, 1976; Olson and Palmer, 1976). The lower levels of selenium in Cozzi’s basal diet were borderline deficient and would have minimal influence on induction of glutathione peroxidases (GSHpx-1 and GSHpx-4). In Juniper’s study, the basal selenium content of 0.16 mg/kg diet was at a concentration where GSHpx-4 would have been approaching maximised activity with an increased sodium selenite and certainly diet. The implication of increased oxidative instability resulting from feeding sodium selenite is that meat quality parameters would probably decrease in refrigerated meats, and the parameters affected include meat colour and rancidity. No data were presented to compare the response to organic selenium.

Medeiros et al. (2012) fed organic selenium (0 to 0.6 mg/kg diet) from the cell walls of Candida pelliculosa to broiler chickens from hatch to 42 days of age. Their results indicated that there was a significant improvement in tenderness of the breast meat as indicated by a significant linear decrease in shear force of the breast meat.

Trials run by Boiago et al. (2014) supplemented broiler diets with either sodium selenite or selenomethionine and examined meat at seven days storage time. They found that breast meat from selenomethionine fed broilers had lower thiobarbituric acid reactive substances (TBARS) concentration than breast meat from sodium selenite fed broilers. When selenium was fed at 0.5 mg/kg diet, shear force was less than in control, and this was associated with higher breast meat pH in breasts from selenomethionine fed broilers.

Meat quality of Grey geese in response to feeding Sel-Plex™ was examined by Baowei et al. (2011), and reported that it decreased shear force of the breast muscle and elevated antioxidant status.

Juniper et al. (2011) compared high and low dietary levels of Sel-Plex™ to sodium selenite and unsupplemented diets fed to commercial turkeys. The background level of natural selenium in the unsupplemented diet was 0.11 mg/kg diet,
which reflected natural selenomethionine from plant sources. They found no differences among dietary selenium treatments for pH of breast meat, but levels were substantially lower than usually found in broiler chickens. However, breast meat GSHpx activity was elevated in the dietary selenium treatments. In thigh meat, high level of Sel-Plex supplementation increased GSHpx compared to low Sel-Plex and sodium selenite and the unsupplemented group, and the TBARS concentration in the thigh meat was lower in Sel-Plex treatments than in sodium selenite and unsupplemented treatments. The dark meat from the turkey thigh had TBARS concentrations that were roughly four fold greater than that found in breast meat, but the author showed that TBARS declined with increasing tissue selenium content, primarily due to high Sel-Plex feeding, and corresponding elevated GSHpx activity in the thigh meat. While on the surface, these results might appear to be minimal since the most dramatic effects were associated with the higher dietary level of Sel-Plex™, these results are significant to nutritionists because the selenium requirement by turkeys is greater than that of the domestic fowl (Sunde and Hadley, 2010). High dietary Sel-Plex™ delivered greater levels of selenium through selenomethionine than did sodium selenite or the low level of Sel-Plex™. Thus, these results should not be surprising.

Zhan et al. (2007) fed pigs either a control diet with no supplemental selenium (natural plant based organic selenium at 0.045 mg/kg basal feed), a diet supplemented with sodium selenite (0.3 mg/kg feed) or a diet supplemented with selenomethionine (0.3 mg/kg feed). They found that muscle GSHpx activity was elevated by both sodium selenite and selenomethionine compared with the control and that there was a bi-phasic TBARS response in muscles with selenomethionine maintaining significantly lower TBARS than control and sodium selenite, which was associated with an eight fold increase in muscle TBARS. The pH of the loin was not altered significantly by the dietary treatments. The redness of the meat was increased by the selenomethionine treatment but not by sodium selenite treatment. These results strongly suggested that sodium selenite, due to its pro-oxidant properties, has the potential to decrease meat quality associated with increased oxidative instability.

**Vitamin E, glutathione peroxidase, and lipid stability related to meat quality**

Lipid oxidation has a detrimental effect on the sensory qualities of stored meat and meat products, making the meat unacceptable to consumers due to rancid or off-flavours (Ladikos and Lougovois, 1990). Lipid oxidation in meat poses a risk to the health of consumers of meat products (Tappel and Dillard, 1981; Pearson et al., 1983; Frankel, 1984; Sanders, 1987). Phospholipids, composed primarily of unsaturated lipids, are more labile than neutral lipids. Oxidation and degradation of polyunsaturated fatty acids is a major contributor to the development of off-flavours (Igene and Pearson, 1979; Renerre et al., 1996). Lipids in poultry exhibit a higher degree of unsaturation than red meats due to the higher content of unsaturated lipids as phospholipids located in membranes (Igene and Pearson, 1979). DeVore et al. (1983) reported that GSHpx was increased in chicken muscle by dietary selenium and this was accompanied by resistance to post mortem oxidation by phospholipids in the meat from selenium-fed broilers.

Reactive oxygen species are formed during normal metabolism causing damage to cellular lipids, proteins, and nucleic acids (Anundi et al., 1979; Chow, 1979; Combs, 1981; Wefers and Sies, 1983; Tsan et al., 1985; Davies, 1987; Machlin and Bendich, 1987). Diets deficient in lipotropic nutrients such as choline, methionine, and vitamin E exacerbates the peroxidation of lipids (Rushmore et al., 1984; Ghoshal et al., 1988; Dianzani et al., 1991). Biological antioxidants that could be beneficial in decreasing the effects of ROS include GSH, vitamin E, and ascorbic acid (vitamin C) (Tappel, 1962; Chance et al., 1979; McCay, 1985; Machlin, 1991). There are interactions among these antioxidants as they act to protect the cell from the effects of ROS (Horton and Fairhurst, 1987). Similarly, selenium supplementation to diets enriched with unsaturated fats can improve stability of lipids in breast meat and thigh meat (Combs and Regenstein, 1980).

It has been noted that there is a consumer demand for additional n-6- and n-3 fatty acids in the human diet (Kouba and Mourot, 2011). However, the authors clearly indicated that enrichment of animal products with dietary sources of the n-6 and n-3 fatty acids raises the risk for development of lipid peroxidation, which would result in decreased meat quality due to increased rancidity, off flavours, and colour deterioration. Researchers support the use of dietary antioxidants such as vitamin E to avert these aforementioned meat quality problems associated with increased potential for increased oxidation of the elevated polyunsaturated fatty acids in the meat.

Pappas et al. (2011) used graded levels of organic selenium in Sel-Plex™ (0, 0.15, 0.3, and 3.0 mg Se/kg diet)
along with 80 mg vitamin E/kg diet and investigated the influence of the organic selenium on poultry meat long chain polyunsaturated fatty acid composition and oxidative stability. The resultant selenium-enriched meat from the 3.0 mg Se/kg diet was found to have linearly increased long chain polyunsaturated fatty acids in addition to reducing the accumulation of malondialdehyde from oxidised polyunsaturated fatty acids. These observations indicated that there is an important role to be played by organic selenium in the maintenance of meat quality as it interacts with dietary vitamin E in poultry meat.

Kim et al. (2010) examined the effects of graded levels of vitamin E (3.68 mg (control: basal level of vitamin E/kg diet with 0.17 mg Sel-Plex™/kg diet), 18.38, 36.77 and 73.53 mg/kg diet) and the combination of vitamin E (36.77 mg/kg diet) with Sel-Plex™ (0.3 mg/kg diet) on oxidative stability of stored chicken thigh meat. The TBARS values increased in all stored meat samples. Increasing dietary levels of vitamin E with basal levels of selenium (0.17 mg/kg diet) was found to decrease TBARS values in thigh meat stored for 10 days compared to the control with no supplemental vitamin E and selenium. The supplementation of additional selenium as Sel-Plex™ reduced TBARS values in stored thigh meat compared to control and all vitamin E-only supplemented diets. Supplementation of Sel-Plex™ to the 73.53 mg vitamin E/kg diet caused a further decrease in TBARS values in stored thigh meat compared with all other vitamin E and Sel-Plex™ only supplemented diets. These observations demonstrated three important effects of vitamin E and Sel-Plex™ on oxidative stability of stored chicken thigh meat. First, at some level between 3.68 and 18.38 mg/kg diet, vitamin E alone is a powerful antioxidant that can reduce the TBARS values in stored meat. Second, Sel-Plex™, at a minimum of 0.3 mg/kg diet, also serves as a very powerful antioxidant in stored chicken meat. Third, the interaction between vitamin E, even at low dietary levels, and Sel-Plex™ has additive effects on the preservation of quality of stored meat via reduction in TBARS values. Similar conclusions were made by Edens (1996), who observed that 12.13 mg vitamin E/kg diet was not as effective as 24.26 mg vitamin E/kg diet in combination with Sel-Plex™ in preserving water holding capacity (WHC) in stored breast meat.

Vitamin E is recognised as the primary lipid soluble antioxidant in membrane lipids and as a protector of associated proteins (Tappel, 1962; Wefers and Sies, 1988; Machlin, 1989, 1991). It has also been postulated that vitamin E has a structural role in membrane stabilisation and permeability (Lucy, 1972). Vitamin E acting in concert with selenium incorporated into the membrane residing GSHpx-4 and cellular GSHpx-1 improves the antioxidant status of chickens and provides a high degree of protection for unsaturated membrane and cytosolic lipids from peroxidative degradation (Combs and Scott, 1974, 1977).

Combs and Regenstein (1980) observed that frozen meat from selenium-fed chickens had lower indices of oxidation than controls, and DeVore et al. (1983) reported that in breast and thigh meat from selenium-fed broiler chickens, higher GSHpx activity was associated with lower TBARS values (Figure 1). Meat quality appears to be a reflection of pre-slaughter GSHpx status in food animals and oxidative stability in stored meat (Renerre et al., 1996). It was of interest that GSHpx activity was significantly less in breast compared with thigh meat, but TBARS values were nearly identical and significantly less in breast and thigh meat from selenium-fed broilers. The differences in GSHpx activity in breast and thigh meats might be indicative of more phospholipids in thigh meat similar to the condition in meat from beef muscles (Mercier et al., 1995).

Sodium selenite was initially the only allowed source of supplemented dietary selenium, and its use improved antioxidant GSHpx activity leading to lower oxidative activity in stored meat. Daun et al. (2004) noted that the oxidative meat found in the thigh of poultry species had greater GSHpx activity than glycolytic meat breast, and that duck meat had significantly greater GSHpx activity compared to the other species.

**Figure 1.** Influence of sodium selenite feeding on chicken breast and thigh muscle raw patties stored at 4 °C for four days on glutathione peroxidase (GSHpx) activity and thiobarbituric acid reactive substances (TBARS) values (adapted from DeVore et al., 1983).
activity than all other poultry species meats. There is a significant positive correlation between GSHpx activity and soluble selenium content in pork and beef similar to that found in poultry meats (Daun and Åkesson, 2004).

An investigation by Wang et al. (2011) examined the effectiveness of sodium selenite, L-selenomethionine and D-selenomethionine on oxidative stability of breast meat and other tissues from broiler chickens that had been fed 10 mg vitamin E/kg of diet. In all tissues and in breast muscle from broilers fed sodium selenite, malondialdehyde concentrations were significantly increased compared with those fed either L-selenomethionine or D-selenomethionine. Between the two isomers of selenomethionine, in all tissues and breast muscle L-selenomethionine was more efficient in increasing total antioxidant capacity, GSH concentration, and GSHpx-1 activity and decreasing malondialdehyde concentrations than either D-selenomethionine or sodium selenite. Both selenomethionine isomers reduced drip loss more than sodium selenite, but neither Hunter a* values nor pH were affected by any of the selenium treatments. Even with a relatively low dietary vitamin E level (10 mg/kg diet), the L-selenomethionine isomer was effective by inducing greater oxidative stability and quality.

Skřivan et al. (2012) examined the influence of vitamin C and selenium (Sel-Plex™ and sodium selenite vs. no supplemental selenium) on the composition and oxidative stability of broiler thigh meat. Selenium supplementation did not affect water content in thigh muscles. Vitamin E content of the muscle was increased by the dietary supplementation of Sel-Plex™ compared to sodium selenite and control treatments, and selenium content of the thigh muscle was increased by the dietary supplementation of Sel-Plex™ compared to sodium selenite and control treatments. Sparing of vitamin E by selenium in guinea pigs (Bertinato et al., 2007) appears to be a viable explanation for the data reported by Skřivan et al. (2012). Selenium supplementation increased GSHpx activity in thigh meat compared to the control fed group, but there was no difference between selenium treatments. In meat stored for five days, dietary supplementation with Sel-Plex™ compared to sodium selenite and control treatments resulted in lower TBARS values, but there was an interaction with dietary vitamin C which allowed for slightly higher TBARS values in meat from Sel-Plex™-treated animals. The observations made in this study supported the concept that selenium improved meat quality via oxidative stability, and that organic selenium was more effective than sodium selenite, possibly through increased levels of vitamins E and C.

Grau et al. (2001ab) studied the influence of the dietary fat source, α-tocopherol (vitamin E at 0 or 225 mg/kg diet), and vitamin C (0 or 110 mg/kg diet) supplementation on oxidative stability of raw and cooked dark chicken meat that was vacuum-packaged and stored at −20 °C for 0, 3.5, and 7 months. The supplementation of α-tocopherol was found to decrease TBARS values in stored and cooked meat. In contrast, vitamin C supplementation provided no protection, and there was no synergism between α-tocopherol and vitamin C supplementation. Polyunsaturated fatty acid-enriched diets increased raw and cooked meat susceptibility to oxidation, but α-tocopherol protected it via lower rates of TBARS accumulation. There was a highly significant negative correlation among the α-tocopherol content of meat and all measures of oxidative deterioration.

Vitamin E plays a key role in cellular stability. The fat-soluble vitamin E (α-tocopherol) is an isoprenoid compound that can be stored in large quantities in the body, with small traces persisting for long periods of time. Vitamin E used as a dietary supplement can be either labile, and oxidation can be accelerated by heat, moisture, rancid fat, copper, and iron. Vitamin E is obtained primarily from dietary sources such as vegetable oils, cereal products containing oils, eggs, liver, legumes, and green plants. Vitamin E exists in eight different forms: four tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ). The difference between tocopherols and tocotrienols is due to saturated side chains of the tocopherols and unsaturation of the tocotrienols.

Dietary supplementation with antioxidants is an effective way to maintain lipid stability in meat (Grau et al., 2001a), and vitamin E, as a major lipid-soluble antioxidant, has the potential to stabilise the membrane-bound lipid against metmyoglobin/H₂O₂-initiated oxidation (Buckley et al., 1989). Vitamin E, interacting with GSHpx-4, reduces fatty acyl hydroperoxy radicals (ROO−) and both phospholipid- and cholesterol-hydroperoxides in cell membranes (Ursini et al., 1985; Thomas et al., 1990).

Vitamin E is recognised as the primary lipid soluble antioxidant in membrane lipids and as a protector of associated proteins (Tappel, 1962; Wefers and Sies, 1988; Machlin, 1991). Vitamin E, when acting as an
antioxidant, can be rendered inactive by any oxidative process. Thus, vitamin E is provided as an esterified commercial supplement in the form of dl-α-tocopheryl acetate to improve its stability. It has also been postulated that vitamin E has a structural role in membrane stabilisation and permeability (Lucy, 1972). When acting in concert with selenium, it improves the antioxidant status of chickens and other animals and provides a higher degree of protection for unsaturated membrane and cytosolic lipids against peroxidative degradation (Combs and Scott, 1974, 1977).

Vitamin E has been shown to have many different functions despite the fact that Donaldson (2001) suggested that vitamin E activity is confined to detoxifying peroxides and free radicals produced from unsaturated fatty acids in membranes. If peroxides accumulate in tissues, free radicals will induce a cascade effect on production of more peroxides from fatty acids within cell membranes. Vitamin E can block that cascade effect, but additional nutrients are required. These include niacin (NADPH from glucose metabolism), riboflavin (for glutathione reductase redox activity), selenium (for glutathione peroxidase activity), vitamin C (reduced by Se-dependent thioredoxin reductase that supplies reducing equivalents for reduction of α-tocopheroxyl to α-tocopherol), and GSHpx4. Other functions of vitamin E relate to a role in membrane structure and prostaglandin synthesis (Ullrey, 1981), blood clotting (Machlin, 1984, 1989), immune response through interaction with selenium (Tengerdy and Brown, 1977; Nockels, 1979), electron transport, DNA synthesis and detoxification of metals such as silver, arsenic, lead, cadmium and mercury via interaction with selenium (Whanger, 1992).

The role of GSHpx in maintenance of meat quality has been discussed above, from which one can conclude that GSHpx-1, GSHpx-4, GSH, and vitamin E are all necessary for maintenance of oxidative stability in muscle cells and in the maintenance of meat quality. However, it is the unique relationship between GSHpx-4 and vitamin E in membranes that plays a pivotal role in the maintenance of meat quality. Among the different GSHpx species, only GSHpx-4 resides in the cell membrane (Ursini et al., 1985). In plasma membranes, the GSHpx-4 interaction with GSH and vitamin E allows for nearly complete inhibition of lipid oxidation (Ursini and Bindoli, 1987). Furthermore, GSHpx-4 activity, which prevents free radical generation from lipid hydroperoxides, can influence the vitamin E requirement necessary to inhibit lipid peroxidation (Ursini and Bindoli, 1987). It is the interaction among GSH, GSHpx-4 and vitamin E in the cell membranes that supports maintenance of meat quality associated with lipid oxidation, odour, colour and even water holding capacity related to cell membrane integrity and protein integrity in muscle fibres.

The influence of Sel-Plex™ on meat quality as presented above is positive, but relatively little has been done to address the interaction of dietary vitamin E and Sel-Plex™ on meat quality. In earlier work (Edens, 1996; Upton et al., 2008) using vitamin E at 24.26 mg/kg diet and selenium as either sodium selenite or Sel-Plex™ at 0.1 and 0.2 mg/kg diet were provided to boilers, it was determined that organic selenium improved meat quality.

These observations strongly suggest that the organic selenium in Sel-Plex™ is superior to sodium selenite in the maintenance of meat quality, but the exact role played by GSHpx-4 in this process remains to be elucidated. This is important as GSHpx-4 interaction with GSH and vitamin E in cell membranes allows for nearly complete inhibition of lipid oxidation via prevention of free radical generation from lipid hydroperoxides in lipoproteins and other complex lipids associated with cholesterol, cholesteryl esters and phospholipids in conjunction with α-tocopherol (Ursini and Bindoli, 1987; Maiorino et al., 1991; Liang et al., 2007). This can reduce the vitamin E requirement (Ursini and Bindoli, 1987).

The mechanism whereby selenium and vitamin E interact to reduce oxidative damage to muscle cells appears to be unequivocal, but there are still many questions that require answers. Apart from the selenium by vitamin E interaction in the maintenance of meat quality, a large knowledge base has been amassed describing the influence of vitamin E on meat quality in poultry, but without addressing selenium involvement (Fellenberg and Speisky, 2006). It has been reported that vitamin E is more efficient than spice extracts in reducing lipid peroxidation in meat from broilers (Lopez-Bote et al., 1993a). Sheehy et al. (1993a) observed that the precursor for vitamin E, α-tocopherol supplementation to poultry feeds increased shelf life of meat as indicated by reduced lipid peroxidation in the meat. Bartov and Frigg (1992) reported that high levels of vitamin E (73.53 to 110.3 mg/kg feed) were negatively associated with TBARS in poultry meat, i.e. vitamin E reduced membrane lipid peroxidation.

Maraschiello et al. (1999) noted a negative relationship between GSHpx-1 activity and α-tocopherol levels in chicken meat, but a positive relationship between
TBARS values and GSHpx-1 activity suggested that cytosolic GSHpx-1 activity could be used as an indicator of fresh chicken meat oxidative stability. The source of dietary selenium was not stated in this work, but it was assumed that sodium selenite served as the source for inorganic selenium. It was noted that GSHpx-1 activity was influenced by the degree of dietary fat saturation with decreasing activity associated with fats from lard, sunflower oil and olive oil, respectively. The presence of \( \alpha \)-tocopherol at 200 mg vitamin E/kg diet did not alter GSHpx-1 activity within the dietary fat treatments. The same authors reported that vitamin E contributed to improved oxidative stability in both raw and cooked chicken meat. These observations were supported by observations made by Fellenberg and Speisky (2006), who noted that lipids and phospholipids, in cell membranes are extremely susceptible to oxidative damage, especially as the degree of unsaturation increases. Bou et al. (2005) compared the effects of sodium selenite and Sel-Plex™ supplementation on consumer acceptability of poultry meat as influenced by dietary fat sources (animal fat, fish oil, and linseed oil). In their study, vitamin E as \( \alpha \)-tocopherol was held constant at an inclusion level of 100 mg/kg of diet. The assessment of selenium related antioxidant activity via induction of GSHpx activity in meat was not addressed, but they acknowledged that Sel-Plex™ versus sodium selenite has the potential to increase such activity leading to lower TBARS values in stored meat. Poultry meat was stored for 74 days and 18 months before it was cooked and evaluated by taste panel. The results of this investigation did not reveal selenium-related differences in acceptability of the cooked chicken meat, but the TBARS values of meat was lower in meats from selenium-supplemented broilers. They did attribute improved oxidative stability of the stored meat to the dietary \( \alpha \)-tocopherol. In a study with female pigs fed sodium selenite as well as low versus high levels of vitamin E, a similar result was attributed to the feeding of high level of vitamin E which was associated with lower rates of lipid peroxidation with lower TBARS values (Nuernberg et al., 2002).

The effects of vitamin E and selenomethionine, as Sel-Plex™, on oxidative stability of frozen-raw and cooked omega-3 polyunsaturated fatty acids (\( \omega-3 \) PUFA) enriched dark chicken meat were explored by Perez et al. (2010). After a 40 day dietary enrichment with \( \omega-3 \) PUFA, there was an increase in oxysterols from oxidised cholesterol in both frozen-raw and cooked dark meat chicken. Meat frozen for six months had unchanged oxysterol concentration, but meat from dietary vitamin E treatments (147.06 mg E/kg diet) tended to have lower oxysterol concentrations. Cooking-related changes in oxysterols tended to be suppressed by dietary vitamin E treatment, but inconsistent results were found in association with meat from Sel-Plex™-fed broilers. Nevertheless, TBARS values were lower six months frozen-raw meat from dietary vitamin E and Sel-Plex™ treatments alone and in combination, but after 12 months storage, the antioxidant effect of both vitamin E and Sel-Plex™ dietary treatments was no longer effective in maintaining lower TBARS values. It must be acknowledged that the amount of fat in raw poultry products that have been frozen can influence oxidative changes (Ang and Young, 1992). Thiobarbituric acid reactive substances (TBARS as malonaldehyde mg/kg of meat) increased after long-term storage if fat content is above 15 to 20% of the product (Ang and Young, 1992). The relationship between dietary fat on fatty acid composition of broiler meat has been documented extensively (Bartov and Bornstein, 1977a,b and 1978). Generally, the lipid stability of broiler meat will be affected adversely if the degree of unsaturation increases as the result of high fat supplementation in the feed such as that reported by Perez et al. (2010).

The feeding of high levels of vitamin E leads to its accumulation in plasma, fatty tissues and in muscle. The accumulated vitamin E then acts to decrease the oxidation of depot lipids. However, if the depot fat is high in unsaturated lipids, there is a limit to the vitamin E influence on stabilisation of depot lipids. In this condition, there would be a need to supplement the diets of broilers with antioxidants to reduce oxidation of dietary lipids and to improve the antioxidant status within the bird itself. As presented above, provision of Sel-Plex™ as a source of organic selenium for incorporation into GSHpx forms, especially GSHpx-1 and GSHpx-4, is the basis for a powerful antioxidant system involving GSH, GSHpx forms, and Vitamin E, which then would interact with GSHpx-4 in membranes to reduce lipid peroxidation that would contribute to improved meat quality, shelf-life of the meat, and reduced development of rancidity due to lipid oxidation. However, the dietary supplementation of antioxidants cannot prevent signs of vitamin E deficiency if levels are low or eliminated from the diet, and this was demonstrated in chickens given organic selenium and vitamin E in difference dietary levels. The need for both vitamin E and selenium, especially natural organic selenium, such as the
selenomethionine, is without question. The need for selenium-based antioxidant enzymes, GSHpx-1 and GSHpx-4, in the maintenance of meat quality is evident, but there is a need to further explore the contribution of GSHpx-4 in the maintenance of poultry meat quality. The interaction of vitamin E with GSHpx, ostensibly GSHpx-4 in cell membranes, will result in decreased concentrations of malondialdehyde, the basis for TBARS values, in freshly processed poultry meat (Zhan et al., 2007).

All antioxidants are not equal in their influence on the development of lipid oxidation in depot lipids and in muscle lipids. As an example, BHT supplementation in the diet was sufficient to stabilise depot lipids but not muscle lipid that could only be stabilised with ethoxyquin. Additionally, it is important to understand that the duration of the time of appropriately administered dietary supplements of antioxidants can have a significant impact on the resultant stability of lipids in broiler meat and depot fat.

Early work by Marusich et al. (1975) observed delayed onset of rancidity of breast meat when broilers were fed vitamin E at a level of 40 mg/kg for eight weeks or 160 mg/kg for the final five days before slaughter. Similarly, feeding vitamin E at 200 mg/kg for four weeks or 400 mg/kg for three weeks before slaughter provided optimal delays in the onset of rancidity in turkey breast. Jensen et al. (1995) reported that a mixture of natural source RRR-α, γ, δ-tocopherol (72 mg/kg α-tocopherol and 69 mg/kg γ-tocopherol) was less effective in protecting broiler meat from oxidative damage than the synthetic all-rac-α-tocopherol acetate (100 mg/kg).

The stability of poultry muscle and adipose tissue can be influenced negatively by thermally oxidised lipids in the diets. Sheehy et al. (1993b) noted that fat blends for animal feeding may be based upon waste fats from frying processes or by-products such as distillation residues from edible oil refining. Oxidised dietary lipids not only reduce the growth rate of broilers but are usually low in antioxidants such as vitamin E. Feeding oxidised safflower oil, sunflower oil, linseed oil, oleic acid, or linoleic acid induced signs of vitamin E deficiency as manifest by development of encephalopathy despite the presence of the antioxidant BHT. This resulted in the reduction of vitamin E concentration in breast and thigh meat and made these meats more susceptible to peroxidation. Feeding diets supplemented with vitamin E at a level of 50 mg/kg only partially stabilised the thermally oxidised lipid induction of depot lipid peroxidation.

Supplementation of vitamin E at a level of 100 mg/kg of diet alleviated tissue lipid peroxidation (Asghar et al., 1989). Later, Galvin et al. (1997) reported that supplementation of vitamin E at dietary levels of 200 to 400 mg/kg may be necessary to achieve optimum muscle α-tocopherol concentrations to offset the negative influence of feeding thermally oxidised sunflower oil. These observations strongly suggest that caution must be exercised in the use of thermally oxidised lipids in formulations of poultry diets.

Thus, development of off-flavours in poultry meat and decreased quality based on sensory perception can be attributed to the presence of ROS that interact with polyunsaturated fatty acids causing them to become oxidised. Part of the process is catalysed by the presence of ionised iron. Bartov and Kanner (1996) studied the effects of iron and vitamin E on oxidative stability of turkey meat. Turkey meat is susceptible to oxidative rancidity during frozen storage especially in dark meat. Increasing dietary iron from 0 to 500 ppm tended to decrease TBARS values resulting in improved oxidative stability during storage of turkey meat. However, Bartov and Kanner (1996) further demonstrated that injection of iron into processed turkey meat did not improve oxidative stability, but in fact contributed to increased oxidative problems. Ahn et al. (1993) reported that ionised iron injected into raw turkey meat acted as a major catalyst for lipid peroxidation. In ice packed chicken meat, free iron will catalyse oxidation of polyunsaturated fatty acids and make the product unacceptable within a few days. However, the chemical deterioration of the meat may be secondary to bacterial growth when the meat is stored at temperatures higher than −18 °C (Sklan and Tenne, 1984).

It is possible to off-set this oxidative damage to poultry meat by providing diets that can be enriched with vitamin E, vitamin C, selenium, and/or methionine and cysteine. In most of the above-referenced work conducted after 1975 and before 1996, the likelihood of sodium selenite serving as a source of dietary selenium is very high, and some of the problems associated with maintenance of meat quality had to be influenced by the oxidative properties of sodium selenite and even vitamin C.

**Pale, soft and exudative meat from poultry**

Pork producers have identified a Halothane gene that is linked to the pale, soft, exudative (PSE) problem in pork (Bendall and Swatland, 1988), but such a gene linkage has
not been reported in poultry. Van Hoof (1979) indicated that turkey breast muscle is susceptible to a PSE-like syndrome similar to that found in pork. The rate at which pH changes post-mortem in processed meat appears to be involved in the PSE syndrome, and this pH decrease can be influenced pre-slaughter by factors such as struggling and heat stress (known to increase muscle glycolysis), and transportation (live haul and time on the truck) that can contribute to a rapid pH decrease in the processed meat (Froning et al., 1978; Van Hoof, 1979; Barbut et al., 1991; Barbut, 1997a,b). Nevertheless, Sante et al. (1991) presented evidence that the rate of decrease in processed turkey breast meat may be genetically controlled, and they noted that the rate of decline in pH was faster in breast meat from a high performance strain as compared to a slow growing breed. Barbut (1997a,b and 1998) and Van Laack et al. (2000) have shown that light coloured chicken meat tended to have a more acidic pH than darker meat, and had less water holding capacity. In turkeys, the incidence of PSE breast meat ranged between 5% and 40% of all turkeys processed.

Barbut (1997b) pointed out that the poultry grading system used worldwide is based on an aesthetic system where carcass grades are based upon skin tears, bruises, and missing parts, but this system does not consider the functional properties of the meat, i.e. texture and water holding capacity. Light coloured broiler breast meat has a tendency to be PSE meat, and its water holding capacity is significantly less than darker breast meat. Among broilers, the PSE syndrome was estimated to range from 0% to 28% of broilers processed (Barbut, 1997b and 1998; Van Laack et al., 2000).

A clear relationship between selenium nutrition and PSE syndrome has not been established. However, Mahan et al. (1999) noted that loin drip loss, pH, and lightness were not affected by dietary organic selenium in Sel-Plex™ source or level, but there was a trend for higher drip loss and a linear increase in loin paleness when inorganic sodium selenite dietary levels increased. These results indicated that dietary sodium selenite had a detrimental effect on loin quality as reflected by higher drip loss and paler colour.

Evidence presented above in sections dealing with selenium involvement in reduction of drip loss and water holding capacity, pH, and color of chicken meat as influenced by selenium shows that organic selenium supplementation to broiler feed resulted in meat with improved water holding capacity, higher post-mortem pH, and improved meat color compared with the supplementation of sodium selenite, which suggested that there is likely a strong influence of selenium sources on development of PSE in poultry meats.

One report of the influence of selenium sources on the frequency of breast meat with signs that suggested development of the PSE syndrome was published by Upton et al. (2009). Broilers during spring and summer seasons were fed either 0.1 or 0.3 mg/kg diet of sodium selenite or Sel-Plex™. The data from this experiment revealed that Sel-Plex™ was more effective in reducing the frequency of subjectively assessed PSE in broiler breast meat than sodium selenite. The lower level of dietary Sel-Plex™ (0.1 mg/kg diet) was equivalent to or better than the higher inclusion rate of sodium selenite (Figure 2). The seasonal influence on PSE frequency was clearly present showing that the stress of the hot summer growing season increased the frequency. Barbut (2009) investigated post-mortem handling of carcasses and development of PSE in turkeys, reported the highest potential for PSE in turkey toms was during the summer and the lowest potential was during the spring growing periods while autumn and winter had intermediate potential for PSE development.

The involvement of pre- and post-mortem oxidative instability in cell membranes appears to play a significant role in development of the PSE syndrome in poultry meat. Evidence presented in this review suggests that the interaction between selenium and vitamin E is extremely important in suppressing the oxidative instability during both pre- and post-mortem states of the muscles. The natural form of selenium, L-selenomethionine derived

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**Figure 2.** Influence of selenium source (sodium selenite (NaSe) vs. Sel-Plex™ (SP)) on subjective frequency of pale, soft, exudative (PSE) breast meat from male broiler chickens grown during spring and summer growing seasons. Unlike lower case letters above bars in the histogram indicate significant differences among treatment means. (Upton et al., 2009).
from plant-based feed ingredients and from yeast-derived Sel-Plex™, is superior compared to the inorganic forms of selenium fed to broilers in making available a steady-state supply of selenium from muscle cell proteins for insertion into GSHpx-1 and most importantly GSHpx-4 for maintenance of the vital antioxidant glutathione-glutathione peroxidase system interaction with vitamin E in cell membranes. There are many factors that contribute to the development of the PSE in poultry meat and included among these factors are genetic heritage of the bird, nutritional status of the bird, many stressors such as high ambient temperature (Cassens et al., 1975; Upton et al., 2009), stunning methods (Backstrom and Kauffman, 1995), pre-slaughter handling (D’Souza et al., 1998), meat pH and post-mortem cooling rate (Offer, 1991; Ferket and Foegeding, 1994; Lee and Choi, 1999; Wölfel et al., 2002).

Zhang et al. (2011) fed oxidised animal/vegetable oils to broiler chickens and found no alteration in growth performance and feed efficiency. Yet, oxidised animal/vegetable oils did cause deterioration in meat quality as shown by decreased water-holding capacity of refrigerated meat, which is similar to the PSE condition. The investigators hypothesised that the decreased meat quality was related to protein and lipid oxidation in the early post-mortem period. Increased oxidative stress pre-slaughter and decreased accumulation of antioxidants in muscles can result in increased levels of ROS and RNS in post-mortem muscle. Upton et al. (2009) examined the influence of dietary Sel-Plex™ and sodium selenite on broiler response to the presence of dietary peroxidised poultry fat. Elevated GSHpx-1, an indicator of the presence of oxidative stress, was observed in response to dietary inclusion of peroxidised poultry fat. Erythrocyte GSHpx-1 activity was elevated by addition of 3 mEq of peroxidised poultry fat by both sodium selenite and Sel-Plex™ supplements. In birds fed 6 mEq of peroxidised poultry fat, GSHpx-1 activity was lower than in those birds fed 3 mEq of peroxidised fat, suggesting that the selenium-dependent antioxidant system was overwhelmed by the lipid peroxides. When Sel-Plex™-fed birds were given 6 mEq of peroxidised fat, the hepatic GSHpx-1 activity did not show as large a decrease as that seen in sodium selenite-fed birds, suggesting that the selenium from L-selenomethionine in Sel-Plex™ was more available for incorporation into selenium-dependent GSHpx-1 enzyme than was the selenite selenium. The L-selenomethionine in Sel-Plex™, as compared with inorganic selenite selenium, improves the selenium status in broilers because it is stored in protein until liberated by natural turnover of the protein. These results indicate that the dietary selenium supplied as Sel-Plex™ improved the selenium and redox status in broilers, leading to greater resistance to oxidative stress than when the inorganic selenite selenium was fed. The involvement of GSHpx in meat quality has been shown above and appears to have a potential role in decreasing PSE in poultry meat.

Conclusions

Clearly, a consumer driven market will continue to demand that the animal-based food industries provide the highest quality meat, and this will become the basis for competitive positions in the national and international markets. Consumers will reject meats that are perceived to lack tenderness or expected visual or odour qualities. On the other hand, pork and turkey meat occasionally have been described as pale, soft, and exudative (PSE). The PSE issue is complex and must be addressed pre-slaughter via dietary and environmental strategies that will impact the post-mortem muscle protein functionality, specifically the moisture retention capacity of the meat as influenced by rate of pH change. However, chief among causes for concern over meat quality is focused on oxidative changes in lipids resulting in rancid odours and off-flavours, which are problems the consumer associates with the industries inability to provide wholesome, high quality, convenient, pre-cooked meat and poultry products. The processing industry has been evaluating functionality of non-meat substances to improve retained moisture and to improve the stability of lipids against oxidation.

The purpose of this review was to examine the role of Sel-Plex™, which provides organic selenium in yeast protein in the issue of poultry meat oxidation. The interaction between selenium, the basis of many enzymes and proteins with antioxidant properties, and vitamin E, which is well known as an anti-oxidant that has a powerful anti-oxidant function in preventing lipid and polyunsaturated fatty acid oxidation seems to be the ultimate focus, perhaps justifiably. Most researchers have potentially measured the wrong selenium dependent anti-oxidant enzyme in GSHpx-1, as information in this review holds that GSHpx-4, which resides within cell membranes along with vitamin E, is the form that should be measured relative to antioxidant influence by selenium in poultry meat. It is clear from the available literature that neither vitamin E nor organic selenium, provided
in Sel-Plex™ can ameliorate post-mortem meat stability problems when used alone in diets. It is the interaction between Sel-Plex™ (as an inducer of GSHpx-4), GSH, and vitamin E in cell membranes that would be the most efficient means to combat lipid and protein oxidation in poultry meat. Oxidative stability of meat has implications for health status of consumers of the meat. Use of Sel-Plex™ as a reliable source of natural organic selenium, which increases muscle selenium content and increases the potential for improved oxidative stability of the muscle and meat, has positive health impact in consumers of the organic selenium enriched meat products.

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Declaration of interest

Dr. A. E. Sefton is an employee of Alltech, Inc.

References


Sel-Plex™ improves meat stability


