Selenium-vitamin E interactions: does 1+1 equal more than 2?

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Introduction

Animal health depends on many factors; and recently it has been appreciated that diet plays a pivotal role in health maintenance and prevention of various diseases. A term 'nutritional immunomodulation' reflects a special emphasis on diet as an important element in maintenance of animal health. It seems likely that soon nutritional science will move toward development of recommendations for optimal animal nutrition for maintenance of productive and reproductive characteristics of animals in commercial conditions with a specific emphasis on animal health. It is already clear that requirements for many nutrients for immunocompetence are higher than for growth and development. Among many dietary factors antioxidants have a special place, being major players in health-related issues. This concept is mainly based on understanding the detrimental effects of free radicals and toxic products of their metabolism on various metabolic processes.

Free radicals and toxic products of their metabolism

Biologically relevant free radicals are activated atoms or groups of atoms (usually containing oxygen or nitrogen) with an odd (unpaired) number of electrons. Typically, stable molecules (not activated) contain pairs of electrons. In a non-radical compound, all orbits are occupied by two electrons. This electron pairing makes the compound relatively stable. When a chemical reaction breaks the bonds that hold paired electrons together, free radicals are produced. Therefore in a 'free radical' compound, there is a single unpaired electron in the outer orbit. A single excited electron is searching to become part of a paired set and will steal an electron from another nearby atom to accomplish this pairing. During this theft, the original free radical becomes stable while the neighbouring atom, by losing an electron, becomes a free radical itself. This new free radical will then seek out another atom to steal from, creating a chain reaction. This biochemical reaction is somewhat like the effect a billiard cue ball has on the break.

Thus, free radicals contain an odd number of electrons, which makes them unstable, short-lived, and highly reactive. The most dangerous free radicals are the small, mobile, and highly reactive oxyradicals. Most biologically produced free radicals and their active metabolites are combined in a group called 'reactive oxygen species' (ROS). Recently, another group called 'reactive nitrogen species' (RNS) was added to this important list. Once formed, these highly reactive radicals can start a chain reaction damaging proteins, lipids and DNA. These ubiquitous unstable oxygen molecules are the natural waste products that result from breathing and other routine metabolic processes involving oxygen. Therefore free radicals are highly unstable and reactive and are capable of damaging biologically relevant molecules such as DNA, proteins or lipids. Damage to DNA is associated with mutations, translation errors and inhibition of protein synthesis, while damage to proteins causes modifications in ion transport and altered enzyme activity. Polyunsaturated fatty acid (PUFA) peroxidation alters membrane composition, structure and properties (fluidity, permeability, etc) and activity of membrane-bound enzymes (Surai, 2002). As a result of that damage to biological molecules, many

systems in the body would be compromised including growth, development, immunocompetence and reproduction.

Production of free radicals in the body is continuous and inescapable. The basic causes include energy production, the immune system, peroxisomes, cytochrome P450 and other enzymes, the arachidonate pathway and reactions with iron and other transition metals.

ENERGY PRODUCTION

Energy-producing processes in every cell generate free radicals and other ROS as toxic waste continuously and abundantly. Oxygen is used to burn glucose molecules that act as physiological fuel. In this energy-freeing operation, which takes place in the electron-transport chain of mitochondria, 1-3% of oxygen consumed can escape from the system and lead to free radical formation. Therefore, because the efficiency of the energy-producing system is less than 100%, free radicals are thrown off as destructive by-products.

THE IMMUNE SYSTEM

Immune cells (phagocytes) deliberately create various ROS and use them as weapons to kill pathogens (to destroy phagocytized bacteria or virus-infected cells). Chronic infection by viruses, bacteria or parasites results in chronic phagocyte activity, free radical overproduction and consequent chronic inflammation, which is a major risk factor for many different diseases.

PEROXISOMES

Peroxisomes, which are responsible for degrading fatty acids and other molecules, produce hydrogen peroxide as a by-product. Under certain conditions, some of the peroxide escapes degradation by catalase, resulting in its release to other compartments of the cell and increased damage to biological molecules.

CYTOCHROME P450 AND OTHER ENZYMES (e.g. XANTHINE OXIDASE)

Cytochrome P450 enzymes in animals constitute one of the primary defense systems against natural toxic compounds from plants as well as detoxification of various xenobiotics. ROS are produced as by-products of these reactions.

ARACHIDONATE PATHWAY

Synthesis of prostaglandins and leukotrienes from arachidonic acid involves peroxidation reactions and ROS formation.

REACTIONS WITH IRON AND OTHER TRANSITION METALS

In the body most of the iron (Fe) and copper (Cu) are bound to specific proteins, which prevents their participation in free radical formation. However, in stress conditions free Fe or Cu are released and stimulate free radical production by reacting with hydrogen peroxides or lipid hydroperoxides.

It must also be noted that free radical production can be substantially increased due to various internal and external factors. In particular, free radical promoters are abundant in our world including stress, over-exercise, injury, smoking, pollution, ozone, chemotherapy, radiation and certain foods.

To what extent are free radicals produced?

About 10^{12} oxygen (O_2) molecules are processed by each rat cell daily, and the leakage of partially reduced oxygen molecules is about 2%, yielding about 2×10^{10} molecules of ROS per cell per day (Chance *et al.*, 1979). Furthermore, Helbock *et al.* (1998) have shown that the DNA in each cell of a rat is hit by about 100,000 free radicals a day; and each cell sustains as many as 10,000 potentially mutagenic (if not repaired) lesions per day arising from endogenous sources of DNA damage (Ames and Gold, 1997). Some oxidative lesions escape repair, and the steady state level of oxidative lesions increase with age (Ames, 2003). An interesting calculation has been made by Halliwell (1994). He assumed that in mitochondria about 1-3% of oxygen consumed may leak from the electron transport chain, forming superoxide radical, and considered that an adult at rest utilizes approximately 3.5 ml O_2/kg/minute or 352.8 liters/day (assuming 70 kg body mass) or 14.7 moles/day. Therefore, if 1%

makes superoxide, this would be 0.147 moles/day or 53.66 moles/year or about 1.72 kg/year of superoxide radical. In stress conditions this rate would be substantially increased. Clearly, these calculations showed that free radical production in the body is substantial, and many thousand biological molecules can be easily damaged if not protected.

The animal body is under constant attack from free radicals, formed as a natural consequence of the body's normal metabolic activity and as part of the immune system's strategy for destroying invading microorganisms. For the majority of organisms on earth, life without oxygen is impossible. Animals, plants and many microorganisms rely on oxygen for the efficient production of energy. However, they pay a high price for the pleasure of living in an oxygenated atmosphere, since high oxygen concentrations are potentially toxic for living organisms. During evolution living organisms have developed specific antioxidant protective mechanisms to deal with free radicals constantly produced in the cells.

Antioxidant protection

In nature there are hundreds and thousands of various compounds possessing antioxidant properties that are able to react with free radicals. They are fat-soluble (vitamin E and carotenoids, etc.) and water-soluble (ascorbic acid, glutathione, bilirubin, etc.), they can be synthesised in the body (ascorbic acid, glutathione) or be delivered with food/feed (vitamin E, carotenoids, selenium etc.). More importantly, there is a range of antioxidant enzymes synthesised in the body that effectively deal with free radicals, but they require metal co-factors. For example, selenium (Se) in the form of selenocysteine is an essential part of a family of enzymes called glutathione peroxidases (GSH-Px) and thioredoxin reductases. Zinc (Zn), copper (Cu) and manganese (Mn) are integral parts of another antioxidant enzyme family called superoxide dismutases (SOD). Iron is an essential part of the antioxidant enzyme catalase. Therefore, only when these metals are delivered in the diet in sufficient amounts can the animal body synthesise these antioxidant enzymes. In contrast, deficiency of those elements causes oxidative stress and damage to biological molecules and membranes.

It is important to remember that all antioxidants in the body work in concert to provide antioxidant defence. In this 'team' one member helps another one work efficiently. Therefore if relationships in this team are effective, which happens only in the case of a balanced diet with sufficient provision of dietary antioxidant nutrients, then even low doses of antioxidants such as vitamin E could be effective. On the other hand, when this antioxidant team is subjected to high stress conditions, free radical production is increased dramatically. During these times, without external help it is difficult to prevent damage to major organs and systems. This 'external help' is dietary supplementation with increased concentrations of natural antioxidants. For nutritionists and feed formulators it is a great challenge to understand when the internal antioxidant team in the body requires help, how much of this help to provide and what the economic return will be.

Let's consider the major points about antioxidant defence that aid the nutritionist in making the right decision. In this regard several points are of great importance. First, there is a delicate balance between the amount of free radicals generated in the body and the antioxidants needed to provide protection against them. An excess of free radicals, or lack of antioxidant protection, can shift this balance resulting in oxidative stress. Oxidative stress plays a major role in many degenerative pathologies and free radical formation is considered a pathobiochemical mechanism involved in the initiation or progression phase of various diseases. In fact, it is widely believed that most human and probably animal diseases at different stages of their development or progression are associated with free radical production and metabolism.

Since the superoxide radical is the major radical produced in physiological conditions (in the cell), the first level of antioxidant defence is based on the activity of superoxide dismutases, a family of enzymes responsible for converting superoxide radical into hydrogen peroxide. The next part of this level of defence is based on the activity of two other antioxidant enzymes, GSH-Px and catalase, which are responsible for conversion of hydrogen peroxide to water. Metal-binding proteins are responsible for preventing appearance of Fe and Cu in free catalytic form, and also belong to the first level of antioxidant defence. Recently it was shown that another Se-dependent enzyme, thioredoxin reductase, also belongs to the first level of antioxidant defence.

Since the first level of antioxidant defence cannot

completely prevent free radical formation and lipid peroxidation, the second level of antioxidant defence has the job of prevention and restriction of chain reactions. They do this mainly by scavenging free radicals and converting them to less reactive compounds. For example, when vitamin E reacts with peroxyl radical, hydroperoxide is produced and vitamin E is oxidised:

Vitamin E + ROO* = ROOH (hydroperoxide) + Vitamin E (oxidized)

In such reactions antioxidants are oxidized and can loose their activity. Due to recycling reactions, the oxidized antioxidants can be returned to active form. This explains why it is so difficult to produce vitamin E deficiency in adult animals. Surprisingly enough, vitamin E, well known as a major antioxidant in biological systems, performs only half a job in preventing lipid peroxidation by converting a free radical into hydroperoxide. In fact, lipid hydroperoxides are still toxic and in presence of transition metals (Fe or Cu) can generate a range of powerful free radicals. The main enzyme responsible for detoxification of the hydroperoxide is selenium-dependant GSH-Px. Vitamin E and Se work in a tandem; and even very high doses of dietary vitamin E cannot replace Se which is needed (in the form of GSH-Px and thioredoxin reductases) to complete the second part of antioxidant defence as mentioned above. Therefore, Se-dependent GSH-Px is responsible for detoxifying hydroperoxide. Selenium, as an integral part of this enzyme, belongs to the first and second levels of antioxidant defence.

Even the second level of antioxidant defence cannot prevent damage to biological molecules; and some lipids and proteins as well as DNA are damaged. Therefore the third level of antioxidant defence is based on the activity of specific enzymes (lipases, proteases, DNA-repair enzymes etc.), which remove or repair damaged molecules. Among various natural antioxidants, selenium occupies a unique position, providing antioxidant power for many different antioxidant mechanisms.

Selenium and selenoproteins

Selenium participates in various physiological functions as an integral part of a range of selenoproteins. The selenoprotein family includes at least 20 eukaryotic proteins (Kohrle *et al.*, 2000). Expression of individual eukaryotic selenoproteins is characterised by high tissue specificity, depends on selenium availability, can be regulated by hormones, and if compromised contributes to various pathological conditions (Kohrle *et al.*, 2000). Most of the selenoproteins contain a single selenocysteine residue per polypeptide chain (Tujebajeva *et al.*, 2000). In fact, the presence of selenocysteine at the active site of an enzyme increases its activity 100-1000-fold (Burk, 2002).

Glutathione peroxidase (GSH-Px) and thioredoxin reductase (TR) are the most abundant antioxidant Se-containing proteins in mammals (Gladyshev *et al.*, 1998). The best characterised among selenoproteins is the GSH-Px family. In mammals it includes five members. The first member of this family, classical GSH-Px, was described in 1973 (Rotruck *et al.*, 1973; Flohe *et al.*, 1973). The second selenoperoxidase, phospholipid hydroperoxide glutathione peroxidase (PH-GSH-Px) was discovered nine years later (Ursini *et al.*, 1982) and characterised in 1985 (Ursini *et al.*, 1985). Next, plasma glutathione peroxidase (PL-GSH-Px) was described in 1987 (Maddipati and Marnett, 1987; Takahashi *et al.*, 1987). The fourth selenoperoxidase, gastrointestinal glutathione peroxidase (GI-GSH-Px), was characterised in 1993 (Chu *et al.*, 1993; for review see Wingler and Brigelius-Flohe, 1999). Recently a fifth member of the Se-dependent GSH-Px family, a specific sperm nuclei GSH-Px (SN-GSH-Px), has been characterised (Behne *et al.*, 2000; Pfeifer *et al.*, 2001). In particular, this selenoenzyme has been identified in rat testes (Behne *et al.*, 2000) to be a 34-kDa selenoprotein. It was localised in the spermatid nuclei and found to comprise about 80% of the total selenium present. It was identified as specific to the sperm nuclei with similar properties to PH-GSH-Px (Pfeifer *et al.*, 2001). The authors showed that it differs from PH-GSH-Px in its N-terminal sequence. In rats, SN-GSH-Px is highly expressed in the nuclei of late spermatids, where it is the only selenoprotein present (Pfeifer *et al.*, 2001). In Se-depleted rats, the concentration of SN-GSH-Px decreased to a third of the normal level and chromatin condensation was severely disturbed (Pfeifer *et al.*, 2001).

The various GSH-Px enzymes are characterised by different tissue specificities and are expressed from different genes (Ursini *et al.*, 1997; Brigelius-Flohe, 1999; Cheng *et al.*, 1998a). For example,

GSH-Px1 is expressed independently of GSH-Px3 or GSH-Px4 and represents approximately 60% of the total hepatic selenium in Se-adequate mice (Cheng *et al.*, 1997). Furthermore, overexpression of GSH-Px1 gene in mice was tissue specific and did not affect the expression of GSH-Px3, GSH-Px4 or GST and plasma selenium levels (Cheng *et al.*, 1997a).

The major function of these peroxidases is the removal and detoxification of hydrogen peroxide and lipid hydroperoxides (Mates and Sanchez-Jimenez, 1999; Ursini *et al.*, 1997). Since hydrogen peroxide is considered an intracellular messenger (Rhee, 1999), and redox regulation can play a basic role in the activation of key transcription factors (Jackson *et al.*, 1998; Dalton *et al.*, 1999), it has been suggested that regulation of the delicate regional redox balance is one of the main functions of GSH-Px enzymes (Brigelius-Flohe, 1999). In contrast, the main function of SN-GSH-Px is protamine thiol cross-linking during sperm maturation (Pfeifer *et al.*, 2001). GSH-Px enzymes are found in all mammalian tissues in which oxidative processes occur (Kohrle *et al.*, 2000). In general, the cytoplasmic GSH-Px is considered an 'emergency enzyme' (Kohrle *et al.*, 2000) responsible for preventing detrimental effects of oxidative stress. GSH-Px enzymes are involved in such physiological events as differentiation, signal transduction and regulation of pro-inflammatory cytokine production (Ursini, 2000). Peroxynitrite scavenging by GSH-Px could also play a prominent role in cell signal transduction events (Sies *et al.*, 1997). Participation of GSH-Px enzymes in regulating biosynthesis of leukotrienes, thromboxanes and prostaglandins is responsible for the modulation of inflammatory reactions, whereas PH-GSH-Px can bring about cytokine-induced transcriptional gene activation (for review see Kohrle *et al.*, 2000).

In general, different forms of GSH-Px perform their protective functions in concert, with each providing antioxidant protection at different sites of the body. For example, GI-GSH-Px could provide a barrier against hydroperoxide resorption in the gut (Brigelius-Flohe, 1999). Recently it was suggested that the digestive tract is a major site of antioxidant/pro-oxidant interaction in the body (Surai, 2002a). In this case a specific GI-GSH-Px could be a major protective factor against lipid hydroperoxides found in the food/feed. It is generally accepted that during feed production and storage some polyunsaturated lipids are oxidised, which can cause health-related problems including decreased growth, productive and reproductive traits of animals and immunocompetence (Kanazawa and Ashida, 1998a,b). It seems likely that this detrimental effect of feed peroxides would ultimately depend on the activity of GI-GSH-Px. Oxidised lipids can react with transition metals, which can be found in the feed as feed supplements (usually in inorganic catalytic form), producing free radicals. Those free radicals can react with natural or synthetic antioxidants present in the feed to form lipid hydroperoxides. Therefore, GI-GSH-Px would prevent those peroxides from entering blood circulation. Indeed, it has been shown that after inclusion of lipid peroxides in diets fed to rats, their concentration in plasma was extremely low (Kanazawa and Ashida, 1998; 1998a). Furthermore, in the gastrointestinal tract there are at least three more selenoproteins including PL-GSH-Px, selenoprotein P and thioredoxin reductase (Mork *et al.*, 1998). As mentioned previously, GSH-Px is an important antioxidant in plasma, which together with selenoprotein P and other antioxidant compounds maintain antioxidant protection. On the other hand, PH-GSH-Px is an important antioxidant inside biological membranes where lipid peroxidation occurs and lipid hydroperoxides are formed.

GSH-Px activity ultimately depends on selenium provision in the diet. However, some forms of GSH-Px are only synthesised when the selenium supply is optimal. There are substantial differences among different forms of GSH-Px with regard to response to selenium deficiency (Brigelius-Flohe, 1999). The selenoproteins retained in tissues for longer periods during progressive selenium deficiency are considered to have higher physiological significance in comparison to those whose activities rapidly decline. In this respect, the main GSH-Px forms rank as follows (Brigelius-Flohe, 1999):

1. Gastrointestinal GSH-Px
2. Phospholipid hydroperoxide GSH-Px
3. Plasma GSH-Px
4. Cytosolic GSH-Px.

Despite great attention focused on the GSH-Px family, it seems likely that thioredoxin reductase, newly described as a selenoenzyme (Gladyshev *et al.*, 1998; Tamura and Stadtman, 1996), may be of even more central importance than GSH-Px.

Recently it has been appreciated that the redox status of the cell is a major determinant of many different pathways including gene regulation (Morel and Barouki, 1999). A thiol redox system consisting of the glutathione system (glutathione/glutathione reductase/glutaredoxin/glutathione peroxidase (Holmgren, 1989; Cotgreave and Gerdes, 1998) and a thioredoxin system (thioredoxin/thioredoxin peroxidase/thioredoxin reductase) are believed to be the major players in this regulation (Holmgren, 2000a,b). Together they supply electrons for deoxyribonucleotide formation, antioxidant defence and redox regulation of signal transduction, transcription, cell growth and apoptosis (Mustacich and Powis, 2000; Arner and Holmgren, 2000). Interestingly, TR can reduce not only thioredoxin, but also oxidized glutathione (Sun *et al.*, 2001). Therefore these two systems are linked much more closely than previously considered. Experiments with yeast mutants lacking both the mitochondrial thioredoxin system and the mitochondrial peroxyredoxin system suggest an important role for thioredoxin, TR and peroxyredoxin in protection against oxidative stress (Miranda-Vizuete *et al.*, 2000). In fact, the TR family is involved in thyol redox regulation, DNA synthesis, regulation of gene transcription and regulation of growth (Burk, 2002).

Furthermore, iodothyronine deiodinases, a family of three enzymes, are responsible for the maintenance of thyroid hormone homeostasis. There is also a range of other selenoproteins identified but their functions are less obvious (Holben and Smith, 1999; Burk and Hill, 1999). For example, sperm capsule selenoprotein (SCS) is localised in the midpiece of the spermatozoa where it stabilizes the integrity of the sperm flagella (Brown and Arthur, 2001) and has been identified as PH-GSH-Px (Ursini *et al.*, 1999). However, recently it has been shown that the pertinent genes of rats and mice did not contain any TGA codons within the translated regions and, as a result, the inclusion of SCS in the family of selenoproteins was questioned (Kohrle *et al.*, 2000). The relationship of various selenoproteins to poultry production is shown in Figure 1.

Selenium-vitamin E interactions: antioxidant properties and beyond

From information presented above it can be

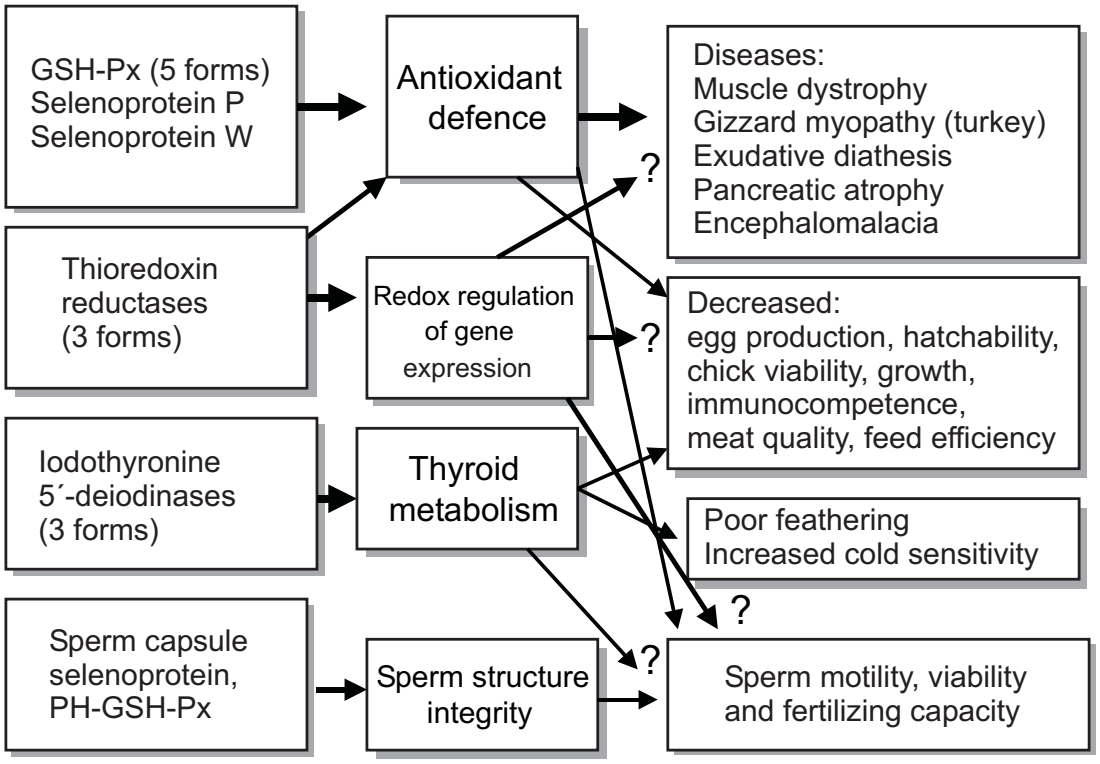

Figure 1. Selenoproteins in poultry production (Adapted from Surai, 2002).

concluded that selenium is a key element in antioxidant defence. Further, selenium works in close concert with other antioxidants, in particular with vitamin E. In fact, nutritional essentiality of selenium was established on the basis of its interaction with vitamin E. Beneficial effects of dietary selenium were first seen in vitamin E-deficient rats (Schwarz and Foltz, 1957). At that time vitamin E deficiency was well described and later it was shown that selenium could prevent some, but not all, of the symptoms of vitamin E deficiency in various animal species. Some selenium- and/or vitamin E-responsive nutritional diseases in rats are shown in Table 1 (Levander *et al.*, 1995). In broader terms, vitamin E and selenium deficiency is related to many different conditions in farm and laboratory animals where all major organs are affected (Table 2). In many cases a combination of these two compounds is needed to achieve the best effect in their treatment or prevention.

Among various selenium-related disorders and diseases, exudative diathesis (ED) has received the most attention. ED is the disease that appears in chickens deficient in both vitamin E and Se and is characterised by severe oedema due to increased permeability of the capillaries in combination with reduced levels of blood proteins (Kristiansen, 1973). As a result of leakage of blood fluid through the capillaries and from minor haemorrhage in muscles, in the area of the breast under the skin an accumulation of exudate with a protein pattern similar to blood serum or plasma can be seen. Haemoglobin degeneration causes a bluish-green colour of the exudate, which can be seen through the skin. Autopsy findings and histopathological lesions were observed only in subcutaneous tissue

Table 1. Nutritional diseases of rats related to selenium or/and vitamin E deficiency.

	Conditions responsive to dietary	
	Se	Vitamin E
Liver necrosis	+	+
Sparse hair coat	+	
Poor growth	+	
Poor sperm motility	+	
Cataracts	+	
Discoloration in body fat	-	+
Discoloration of uterus	-	+
Depigmentation of incisors	-	+
In vitro haemolysis	-	+
Impaired reproductive capacity of females	-	+

Adapted from Levander *et al.*, 1995

Table 2. Diseases associated with selenium and vitamin E deficiency in animals.

Syndrome	Tissue or organ affected	Species
Encephalomalacia	Cerebellum	Chick, turkey, emu, partridge, quail, pheasant and various zoo species
Exudative diathesis	Vascular	Chick, turkey, duck, salmon, catfish
Microtic anaemia	Blood, bone marrow	Chick, monkey, pig, rat, salmon, catfish
Liver necrosis	Liver	Pig, rat, mouse
Pancreatic fibrosis	Pancreas	Chick, salmon, mouse
Erythrocyte haemolysis	Erythrocytes	Chick, lamb, monkey, rat
Muscular degeneration	Skeletal muscle	Chick, duck, goose, ostrich, flamingo, monkey, dog, rabbit, guinea pig, horse, calf, lamb, kid, mink, antelope, pig, rodents, salmon, catfish
Microangiopathy	Heart muscle	Turkey, pigs, calf, lamb, rat, dog, rabbit, guinea pig, cow, sheep, goat, baboon, antelope, elephant, deer
Kidney degeneration	Kidney tubules	Monkey, rat, mouse
Embryonic degeneration	Vascular system	Pig, rat, mouse
Poor hatchability	Egg embryo	Chick, turkey
Steatitis	Adipose tissue	Pig, chick
Testicular degeneration	Testes	Pig, calf, chick, pig, monkey, rat, rabbit, guinea pig, hamster, dog
Retained placenta	Placenta	Cow
Impaired fertility	Spermatozoa	Sheep, cattle, poultry, pig, rat
Ill-thrift	Thyroid, pituitary	Lamb, calf

Adapted from MacPherson, 1994; Surai, 2002

and skeletal muscle. In particular, the subcutaneous tissue was oedematous with hyaline vascular lesions and haemorrhages (Hassan *et al.*, 1990). The thigh muscles were more susceptible to lesions than were the breast muscles, and showed in acute stages degenerative processes of the muscle fibres including calcium deposits, vascular lesions and haemorrhages. In subacute and chronic cases, reparative changes and muscle damage may develop independently of the hyaline vasculosis (Hassan *et al.*, 1990).

Exudative diathesis can occur at any age, but is most prevalent in young growing chickens and turkeys (Whitehead and Portsmouth, 1989). In chicks obtained from laying hens depleted of vitamin E and selenium, ED was observed at hatching, indicating that the deficiency lesions had developed during the embryonic period. In contrast, these signs were not observed in chicks obtained from commercial laying hens adequate in vitamin E and selenium fed the depletion diets until they were two weeks old (Hassan *et al.*, 1990).

Exudative diathesis will only occur if the diet is deficient in selenium, and selenium is 200 times more effective than vitamin E in preventing this disease. Therefore this symptom is primarily considered a selenium deficiency disease (Machlin and Gordon, 1962). ED is also described in ducklings (Dean and Combs, 1981). ED develops in chickens at age of 3-6 weeks; and chicken mortality can be as high as 80% (for review see Surai *et al.*, 1994; Surai, 2002). ED was associated with low levels of muscle Se, liver Se-GSH-Px and vitamin E and was also accompanied by a simultaneous increase in the liver non-Se-GSH-Px (Hassan *et al.*, 1990). It has been suggested that the inflammatory response associated with Se/vitamin E deficiency in a chick may be responsible for ED (Bartholomew *et al.*, 1998). The authors suggested that cytokines produced by leukocytes could be responsible for transparent fluid accumulation and haemorrhaging. It is well recognised that selenium is the major protection against ED. The supplemental vitamin E level of 15 mg/kg was not adequate to provide complete protection against ED (Hassan *et al.*, 1990). ED was inhibited by the rutin and silymarin treatments, but exacerbated by quercetin, morin and ferulic acid. Changes in concentrations of vitamin E in plasma, liver, or muscle, caused by the various treatments (other than vitamin E), were not related to protection against ED (Jenkins *et al.*, 1992).

As mentioned above, molecular mechanisms of ED development as well as nutritional encephalomalacia (NE) and nutritional muscular distrophy (NMD) are still not clear. However it is generally believed that oxidative stress is a crucial factor in disease development. Indeed, lipid peroxidation, disrupting membrane structure and function, is responsible for specific changes in the brain, muscle and vascular system. In this respect a great body of evidence indicates that vitamin E is effective in preventing NE, NMD and ED. However, effective doses of this vitamin for prevention of the diseases are different; and its combinations with other antioxidants are of great importance. Furthermore, protein oxidation and inactivation is another important factor involved in those pathologies. Based on a concept of an integrated antioxidant system (Surai, 2002) it is feasible to suggest that vitamin E recycling and its interactions with other antioxidants, including selenium, ascorbic acid, glutathione etc. are driving forces in prevention of vitamin E deficiency symptoms. For example, recent understanding of the glutathione and thioredoxin systems in general, and thioredoxin reductase in particular, helps explain many interactions between vitamin E and selenium in preventing NMD and ED. Indeed, the thioredoxin system is responsible for maintenance of proteins in active reduced form and could be considered an important factor in development of NMD. Furthermore, TR takes part in vitamin E recycling via interactions with ascorbic acid, and could be a crucial point in prevention of NE and ED. Clearly there is a need for further research in this fascinating field and a chicken model with specific diseases associated with vitamin E deficiency could be used for further elucidation of molecular mechanisms of antioxidant interactions.

It is somewhat surprising that the most commonly used inorganic seleno-compound, sodium selenite, is capable of promoting superoxide radical formation and oxidative stress through its reductive reaction with reduced glutathione (for review see Surai, 2002; 2002b). In contrast, selenomethionine (SeMet) is a relatively non-toxic, non-catalytic and non-redox selenium compound exhibiting low toxicity which does not produce superoxide (Stewart *et al.*, 1999). In fact, when selenium provided as selenite, pure SeMet, ebselen or Se-yeast were investigated in an *in vitro* LDL oxidation model, it was shown that Se-yeast is a powerful *in vitro* and *in vivo* antioxidant (Vinson *et al.*, 1998). Similarly, treatment of lymphocytes with SeMet prior to adding hydrogen

peroxide inhibited peroxyl radical formation in a manner dependent on SeMet concentration (Sun *et al.,* 1997). Furthermore, SeMet is considered a powerful antioxidant, protective against the damaging effects of peroxynitrite. For example, SeMet protected human fibroblast lysates from toxic effects of peroxynitrite (Sies *et al.,* 1998). Selenomethionine also protected dihydrorhodamine 123 from oxidation and 4-hydroxyphenylacetate from nitration caused by peroxynitrite, while sodium selenite exhibited no effect (Briviba *et al.,* 1996). It should also be noted that the selenoxides can be effectively reduced by glutathione, establishing a biological line of defence against peroxynitrite (Sies *et al.,* 1998; Assmann *et al.,* 1998). The rapid and efficient reduction of the selenoxide to SeMet by glutathione in a stoichiometric reaction utilizing two equivalents of thiol has been described (Assmann *et al.,* 1998). Probably radioprotective and UV-protective properties of SeMet (Schrauzer, 2000) could also be associated with its antioxidant properties. Selenomethionine has also been shown to have protective effects on gap junction communications diminished by peroxynitrite (Sharov *et al.,* 1999). The antioxidant properties of SeMet were also demonstrated in a model system based on an olive oil oxidation process at 303 K and 333 K (Zalejska-Fiolka, 2000).

In addition to aforementioned beneficial effects of SeMet, it seems likely that this selenoamino acid is directly involved in the third line of antioxidant defence. For example, recently it has been shown that SeMet induces a DNA repair response and protects normal fibroblasts from DNA damage (Seo *et al.,* 2002a). The induction of DNA repair occurred at a concentration (10 mM) easily attainable *in vivo* by dietary supplementation. Seo *et al.* (2002b) also showed that selenium in the form of SeMet can activate the p53 tumor suppressor protein by a redox mechanism that requires the redox factor Ref1. In fact, SeMet induced sequence-specific DNA binding and transactivation by p53, indicating a stimulation of DNA repair branch of the p53 pathway.

It is interesting that no evidence for enhancement of repair of methyl methanesulfonate-, UV- or bleomycin-induced DNA damage was observed in human fibroblasts treated with selenite (Snyder, 1987). In fact, selenite was shown to induce DNA strand breaks as measured by two independent assays (Snyder, 1988). Sodium chromate induced DNA breaks in Chinese hamster V-79 cells mediated by the formation of free radicals and/or cellular reductive metabolism; and pre-treatment with sodium selenite enhanced formation of DNA breaks (Sugiyama *et al.,* 1987). Selenate is also able to increase one-electron oxidation of plasmid DNA (Milligan *et al.,* 2002). Selenite is toxic and slightly mutagenic for yeast leading to DNA damage (Pinson *et al.,* 2000). High doses of selenite resulted in cytotoxicity and induction of 8-hydroxydeoxyguanosine (8-OHdG) in DNA of primary human keratinocytes compared with those treated with the same doses of SeMet. In fact, selenite generated 8-OHdG DNA adducts, induced apoptosis and was found to be cytotoxic in mouse keratinocytes. On the other hand, SeMet was not cytotoxic, did not generate 8-OHdG adducts and did not induce cellular apoptosis at any of the selenium concentrations studied (Stewart *et al.,* 1999). Co-incubation of ascorbic acid or Cu sulphate with selenite appeared to protect primary human keratinocytes against selenite-induced cytotoxicity. However, synergistic effects were observed between selenite and trolox resulting in enhanced cytotoxicity. On the other hand, SeMet alone or in combination with vitamin C, trolox or Cu sulphate did not affect cell viability (Shen *et al.,* 2001). Furthermore, selenium-containing compounds can protect DNA from damage caused by peroxynitrite. For example, SeMet and selenocystine protected DNA from single-strand breaks more effectively than their sulphur analogs, methionine and cystine, and they also were more effective than glutathione or the hydroxyl radical scavenger mannitol (Roussyn *et al.,* 1996). Furthemore, SeMet decreased DNA damage imposed by peroxinitrite more effectively (by 77% at 0.1 mM concentration) than selenocystine (by 42% at 1 mM concentration; Epe *et al.,* 1996). The extent of DNA damage in prostate cells and in peripheral blood lymphocytes, as determined by the alkaline comet assay, was lower among Se-supplemented (Se-Met or yeast-enriched with selenium) dogs than among control dogs but was not associated with the activity of the antioxidant enzyme GSH-Px in plasma (Waters *et al.,* 2003).

Therefore it is clear that SeMet has a unique specific effect on the DNA repair system in addition to providing protection against DNA damage. This is not the case when selenite is used; and in fact the opposite can occur. This effect of SeMet is related to the anticancer properties of this compound. Mukherjee *et al.* (2001) suggested

several mechanisms related to these properties of SeMet including acceleration of detoxification of carcinogenic compounds, alteration of hepatic drug metabolism, protection against chromosomal damage, enhancement of DNA-repair processes, strengthening of antioxidant protection and stimulation of immune defence mechanisms.

All aforementioned mechanisms through which SeMet is beneficial are relevant to animal/poultry production, and only some of them can be shared by vitamin E. From information presented above, it is clear that selenium and vitamin E are important elements in maintaining antioxidant system efficiency. An additive or co-operative effect of selenium and vitamin E is shown in chick embryonic development (Surai, 2000), in improvement of meat quality (Surai and Dvorska, 2002a,b), in semen quality (Surai *et al.*, 1998), immunity and mycotoxicosis, among others (Surai, 2002). These interactions are mediated via GSH-Px, PH-GSH-Px, GI-GSH-Px, TR or SeMet itself (Figure 2). It seems likely that there are some specific stress conditions where protective effects of selenium cannot be replaced by vitamin E. For example, GSH-Px provided protection in mice against paraquat-induced lethality, while α-tocopherol (20 mg/kg diet) was not effective (Cheng *et al.*, 1998b). Using GSH-Px1 knockout mice it has been shown that even high vitamin E doses (750 or 7500 mg/kg diet), while inhibiting lipid peroxidation in the liver, were not able to replace the protection of GSH-Px against paraquat-induced lethality (Cheng *et al.*, 1999). In the same study a linear increase in PH-GSH-Px activity in the liver accompanied an increase in dietary vitamin E supplementation. Furthermore, it seems likely that normal GSH-Px expression is necessary to protect mice against lethality and hepatic protein oxidation due to diquat (a herbicide generating superoxide radical) toxicity (Fu *et al.*, 1999). Therefore, even though selenium and vitamin E are working together, there are various separate functions of these two distinctive antioxidants. For a nutritionist looking for the best productive and reproductive characteristics of farm animals, this means that an optimal combination of these two elements is needed. Furthermore, organic selenium provides extra benefits in comparison to traditional selenite; and based on recent findings it seems likely that the full replacement of selenite by organic selenium is necessary.

It has been suggested that antioxidant/pro-oxidant balance in the body is responsible for maintaining human and animal health as well as productive and reproductive performance of farm animals. This balance can be adversely modulated by suboptimal diets and nutrient intakes or positively affected by dietary supplementation. Therefore, feed components can modulate maintenance of this balance and may thereby influence the immune system and disease resistance. Thus, the most

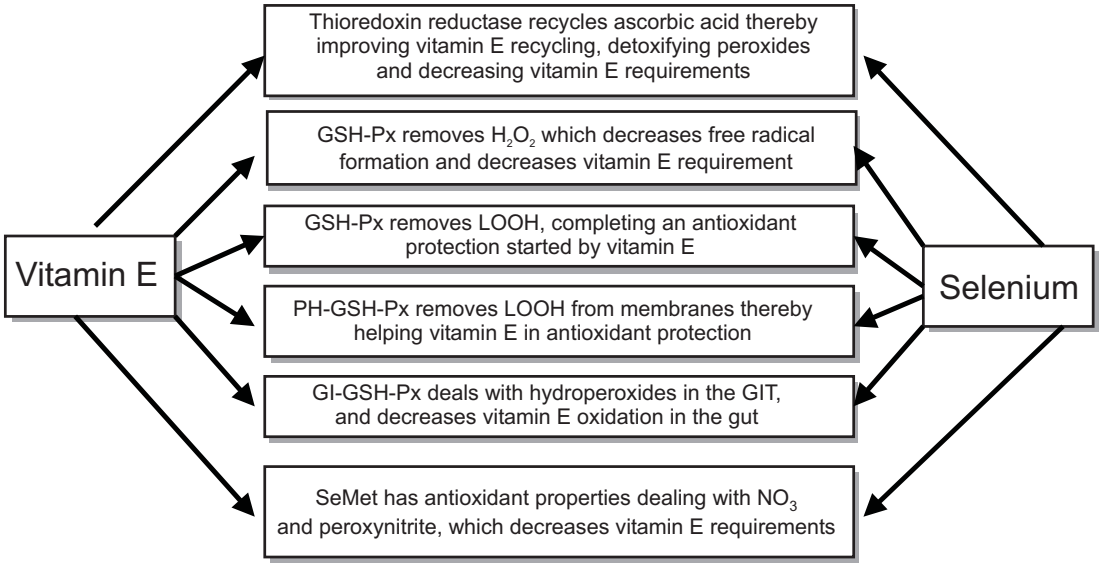

Figure 2. Vitamin E-selenium interactions.

important step in balancing oxidative damage and antioxidant defence in the animal body would be to enhance antioxidant capacity by optimising dietary intake of antioxidants. In this case, selenium and vitamin E play major roles.

Two major applications of natural antioxidants, mainly selenium and vitamin E, in relation to animal production are their roles in semen quality and immunocompetence.

Antioxidants and male fertility

The process of fertilisation of ova is very complex and well regulated. For example, in order to be fertile spermatozoa must have high motility and acrosomal integrity. Furthermore, to be motile they must have intact mitochondria and optimum membrane flexibility and fluidity. Spermatozoa of various animal species have different shapes, but basic structure is similar with the midpiece being the site where all mitochondria are located. Furthermore, to maintain membrane properties there is a high concentration of polyunsaturated fatty acids. In fact, spermatozoa of all animal species have extremely high proportions of those fatty acids (Figure 3). As a result, sperm are very vulnerable to oxidative stress. To protect membrane properties, the antioxidant system of spermatozoa includes fat-soluble and water-soluble chain-breaking antioxidants as well as antioxidant enzymes. A beneficial effect of vitamin E on turkey (Table 3) and goose (Table 4) semen was shown earlier (Surai *et al.,* 1997).

Our data indicated that increased dietary vitamin E supplementation decreased lipid peroxidation in chicken semen; however, the best protection was achieved when vitamin E was combined with selenium (Surai *et al.,* 1998; Figure 4). Understanding the involvement of selenium in maintenance of semen quality came from data on selenoproteins. A specific sperm capsular selenoprotein, recently identified as PH-GSH-Px, is located in the midpiece of spermatozoa. Since mitochondria are the main source of free radicals in the spermatozoa and they are located in the midpiece, antioxidant protection is crucial to sperm motility and therefore fertilizing ability. For example, Se-deficiency causes various sperm abnormalities in this region resulting in decreased fertilising ability; and dietary organic selenium is more efficient in improving semen morphology than selenite. Recently Edens (2002) showed that when cockerels were fed a basal diet containing 0.28 ppm Se without additional dietary supplementation of this trace element, the percentage of normal spermatozoa was only 57.9%. Two major abnormalities seen were bent midpieces (18.7%) and corkscrew heads (15.4%). When this diet was supplemented with an additional 0.2 ppm Se in the form of selenite, the percentage of normal spermatozoa increased to 89.4% and abnormalities decreased. However, when organic selenium (Sel-Plex^{TM} selenium yeast) was included in the cockerel diet in the same amount, semen quality was further improved and those abnormalities decreased further while the

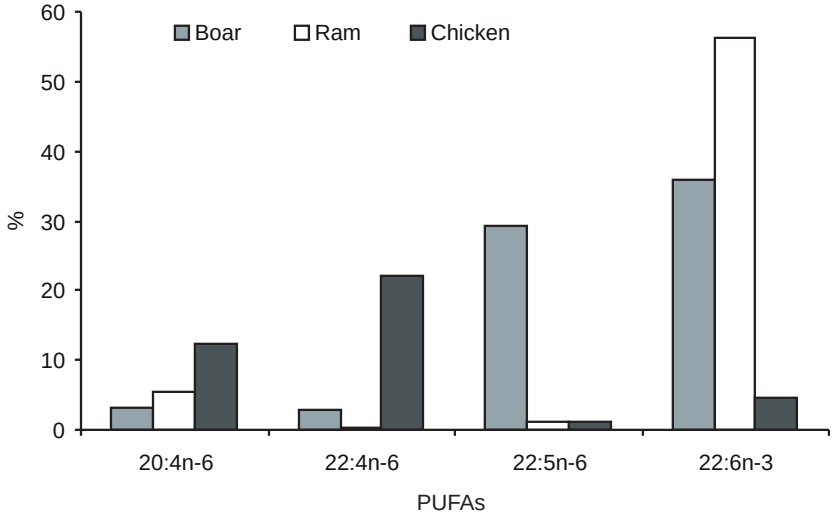

Figure 3. PUFAs (%) in spermatozoa phospholipids.

Table 3. Effect of vitamin E on turkey semen quality.

Group	Vitamin E (mg/kg diet)	Eggs incubated	Fertility (%)	Hatchability of fertile eggs (%)	Hatch from eggs set (%)
1	20	4085	86.4	80.7	69.8
2	60	4104	89.0	82.7	73.6

Surai *et al.*, 1997

Table 4. Effect of vitamin E on goose semen quality.

Group	Vitamin E (mg/kg diet)	Eggs incubated	Fertility (%)	Hatch from eggs set (%)
1	5	2858	92.3	68.8
2	20	3460	94.3	70.1
2	40	4842	95.6	71.4

Surai *et al.*, 1997

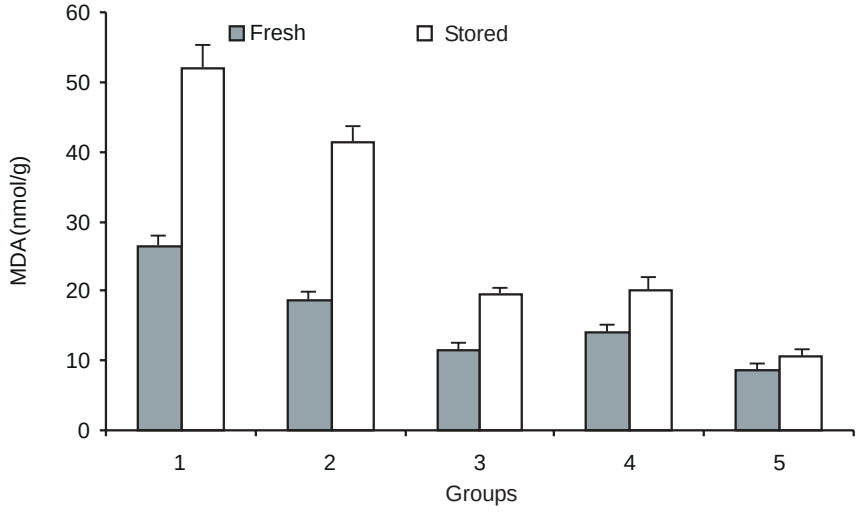

Figure 4. Effect of vitamin E and selenium on lipid peroxidation in fresh and stored chicken semen. 1-no supplementation; 2- 20E; 3-200E; 4- 20E+0.3Se; 5-200E+0.3Se (Surai, 2002).

percentage of normal spermatozoa increased to 98.7%. These results clearly showed that the form of dietary selenium supplementation is crucial to its metabolic efficiency.

Additional data from Edens (2002) indicated that organic selenium from Sel-Plex™ (0.3 ppm Se from 21 weeks of age) in the diet of Hubbard roosters improved semen quality to a greater extent than achieved by selenite at the same dose. The sperm quality index significantly increased as well as the percentage of normal spermatozoa. At the same time, the proportions of various abnormalities in semen decreased. There was also a positive effect on fertility, which was improved by 0.56-1.03% by Sel-Plex™ dietary supplementation. These experimental results confirmed the importance of selenium in maintaining chicken semen quality and specifically showed the advantages of organic selenium. This also aids in explaining improved fertility noted in response to Sel-Plex™ organic selenium (Agate, 2000). The duration of fertility improved; probably due to organic selenium enhancing antioxidant protection in sperm storage tubules (SST, Surai *et al.*, 1998). A unique feature of avian reproduction is the storage of spermatozoa within oviduct SST, which enables the hen to produce fertile eggs during the 'fertile period' of 1-6 weeks, depending on the species (Lake, 1975).

Thus avian spermatozoa might be expected to have systems that will maintain stability throughout this period. Indeed, recent results have confirmed the existence of a complex antioxidant system in the utero-vaginal portion of the fowl oviduct (Breque and Brillard, 2002). In particular GSH-Px activity in the utero-vaginal junction was 12-fold higher than in the liver.

A combination of selenium with vitamin E appears to provide best results in fertility. For example, when cows in a temperate climate received intramuscular injections of vitamin E (500 mg) and selenium (50 mg) 30 days postpartum, the pregnancy rate at second service significantly increased. Reduced services per conception was observed as well as a shorter interval between calving and conception (Arechiga *et al.*, 1998). Thus, injection of vitamin E and selenium increased fertility in cattle that did not become pregnant at first service. Furthermore dairy cows on three New South Wales dairy farms had higher first service conception rates following treatment with oral selenium pellets (McClure *et al.*, 1986).

The effect of combined supplementation with selenium and vitamin E on fertilization of ova was evaluated in beef cattle maintained on either adequate or inadequate planes of nutrition. Fertilization of ova was 100% in females receiving adequate nutrition and supplemental selenium/vitamin E (Segerson *et al.*, 1977). Interaction between plane of nutrition and selenium/vitamin E was significant for percent fertility. When nine oligoasthenoteratozoospermic men were supplemented for six months with selenium and vitamin E, statistically significant increases were observed for sperm motility, percent live and normal spermatozoa. Those improvements were judged supplementation-dependent, since all parameters returned to baseline values during the post-treatment period (Vezina *et al.*, 1996). Recently a significantly positive correlation was observed between human semen selenium concentration and sperm density as well as sperm count, sperm motility and viability (Xu *et al.*, 2001). Moreover, 8-OhdG levels in sperm DNA were inversely correlated with semen selenium concentrations in fertile and infertile subjects.

Antioxidants and immune system

The immune system of the animal is based on natural and adaptive immunity. Natural immunity is dependent on the efficient function of phagocytic cells, namely neutrophils and macrophages, which use free radicals to kill pathogens. However, on escape from the phagosome the same free radicals become dangerous and can damage biological molecules, which compromises phagocyte function and reduces adaptive immunity. Phagocytes also produce communication molecules (eicosanoids, cytokines, etc.), which are used for effective communications between various immune cells.

Adaptive immunity is based on activity of B- and T-lymphocytes, which produce antibodies to specific non-self substances (B-lymphocytes) or directly attach to them (T-lymphocyte) and remove them from the cell. Obviously communication between those cells is a crucial factor in immunocompetence. Major immune cells (macrophages, neutrophils, T- and B-lymphocytes) have receptors on their surfaces that are extremely sensitive to communicating molecules, but they are also sensitive to free radicals and can be easily damaged. It is clear that antioxidant defence of these specialized cells is a key factor determining the effectiveness of the immune system.

There is a great body of information confirming this. Selenium deficiency is associated with compromised functions of natural and adaptive immunity. In particular, phagocytic functions, lymphocyte proliferation and antibody production are compromised (Surai, 2002). On the other hand, selenium and its combination with vitamin E improve immunocompetence and increase resistance to various diseases. This is true for a variety of farm and companion animals as well as humans.

Conclusions

Vitamin E was discovered in 1922 and since then a great body of information has accumulated about its chemistry and biochemistry as well as the major molecular mechanisms of its physiological function. In fact, the image of vitamin E is associated with health, and in animal nutrition there is no doubt about the essentiality of this antioxidant. In contrast to vitamin E, the history of selenium has not been so bright. Selenium is associated with toxicity in the minds of many; and concerns about safety in handling and dosing are always noted. However, in the last several years new findings in selenium biochemistry are changing the 'toxic' image of selenium toward one associated with optimum health

and performance, particularly in response to the growing body of work using organic selenium from yeast. It is clear now that optimal level of selenium in the diet of farm animals is as important as vitamin E. There are several very important advantages of organic selenium in animal nutrition:

- *Accumulation in tissues to provide reserves of this element.* Stored selenoamino acids can be used in stress conditions for additional synthesis of selenoproteins to counteract adverse effects of excess free radicals.
- *More efficient transfer from feed to the egg and embryonic tissues.* Improved antioxidant defence of newly hatched chicks promotes disease resistance and improves chick viability.
- *More efficient (in comparison to selenite) transfer via placenta.* Neonates are born with better antioxidant defense.
- *More efficient transfer to colostrum and milk.* Nursing animals are provided additional antioxidant protection at the critical period of ontogenesis, when antioxidant defence is most crucial;
- *More effective in maintenance of semen quality.*
- *Improved shelf life of meat and eggs.*
- *Less toxic.*
- *Higher selenium content of edible animal products.* More effective transfer to eggs, milk and meat makes organic selenium a preparation of choice for production of Se-enriched animal products, which could be included in the category of functional food.
- *SeMet possesses antioxidant properties on its own while selenite is pro-oxidant.*
- *SeMet has specific functions, probably not related to antioxidants, including a positive effect on DNA-repair enzymes.*

It must be remembered that all antioxidants in the body operate in concert to form an integrated antioxidant system, responsible for protection against damage caused by free radicals and toxic products of their metabolism. Therefore selenium, as a part of various selenoproteins, is a keystone of the antioxidant system. The ability of farm animals to adequately respond to stress conditions depends on the efficiency with which this group of selenoenzymes and other antioxidants interact. Optimal provision of dietary organic selenium in combination with vitamin E is the first most important step in maximizing this efficiency. Improved stress and disease resistance, with enhanced reproductive and productive performance are the result.

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