Selenium in poultry nutrition 1. Antioxidant properties, deficiency and toxicity

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Selenium (Se) has a special place among the feed-derived natural antioxidants, being an integral part of selenoproteins participating in the regulation of various physiological processes in the body. As a part of glutathione peroxidase (GSH-Px) Se belongs to the first and second major levels of antioxidant defence in the cell. There are two major sources of Se for poultry organic selenium, mainly in the form of selenomethionine (SeMet), which can be found in any feed ingredient in varying concentrations and inorganic selenium, mainly selenite or selenate, which are widely used for dietary supplementation. There is a principal difference in metabolism and efficiency of these two forms of selenium, with SeMet being more effective. In fact SeMet possesses antioxidant properties, however, in some conditions selenite can be a pro-oxidant. Se deficiency and excess in modern poultry production are very rare. In general, adequate Se supplementation is considered to be a crucial factor in maintaining the high productive and reproductive characteristics of commercial poultry.

Key words: selenium; selenomethionine; antioxidant; prooxidant; chicken; toxicity

Introduction

Efficient poultry production is based on the feeding of well-balanced diets to highly productive lines of birds. In this respect natural antioxidants play an important role in maintaining bird health, productivity and reproductive characteristics. Vitamin E is widely used in poultry diets; and the level of its supplementation has been increased several-fold during the last few years (Surai, 1999). Recently carotenoids have been included in the antioxidant family, but their precise role in avian nutrition awaits investigation (Surai and Speake, 1998; Surai *et al.*, 2001; 2001a). In general, an integrated antioxidant system has been described in avian tissues (Surai, 1999, 2002); and it has been suggested that the cell's first line of antioxidant defence is based on the activity of three enzymes: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase. In this respect GSH-Px has received only limited attention in relation to poultry production. However, during recent years the importance of this enzyme in the antioxidant protection of tissues has become increasingly appreciated. Since the major form of GSH-Px is Se-dependent, the

role of Se in animal nutrition has attracted considerable attention (Mahan, 1999).

Se is recognised as having anti-carcinogenic and antiviral properties and is known to have important roles in reproductive function and development, immunocompetence and ageing. These Se functions have been described recently in a series of comprehensive reviews (Brigelius-Flohe, 1999; Wingler and Brigelius-Flohe, 1999; Flohe *et al.*, 2000; Kohrle *et al.*, 2000; Arner and Holmgren, 2000; Holmgren, 2000; Schrauuzer, 2000; Whanger, 2000). However, these reviews deal mainly with the general biological and medical issues surrounding Se. In contrast, the majority of publications relating to the role of, and responses to dietary Se in poultry nutrition appeared during the 1970s; and recent understanding of antioxidant system functions and new discoveries regarding the GSH-Px enzyme family are the basis for further development in the Se nutrition of poultry.

Selenium as an integral part of the antioxidant system

The concept of an integrated antioxidant system in the animal cell has developed over the last few years (Halliwell and Gutteridge, 1999; Packer, 1992; Diplock *et al.*, 1998). In relation to poultry, this topic has been addressed in previous reviews on vitamin E (Surai, 1999), Se (Surai, 2000) and carotenoids (Surai *et al.*, 2001). The key points are:

1. Oxygen is an essential element for animal life, but can be toxic in excess.
2. Free radicals are constantly produced in physiological conditions and their production increases in stress conditions.
3. The main sources of free radical production in the cell include the electron transport chain in mitochondria, xenobiotic-metabolising enzymes and immune cells. Specifically, immune cells generate free radicals to kill pathogens.
4. The development of an antioxidant system is an adaptive evolutionary mechanism of survival in an oxygenated atmosphere.
5. The antioxidant system in the cell is based on the three major levels of defence. SOD, GSH-Px, catalase and metal-binding proteins form the first level of defence through prevention of free radical formation. Chain-breaking antioxidants (vitamins A, E, C, carotenoids, glutathione, uric acid etc.) belong to the second level of defence and deal with prevention and restriction chain formation and propagation. A third level of antioxidant defence deals with damaged molecules in the cell as a result of free radical action and toxic products of their metabolism and includes various enzymatic systems responsible for repair or removal of the damaged molecules.

Free radical formation is considered a pathobiochemical mechanism involved in the initiation or progression phase of various diseases including cardiovascular disease, some forms of cancer, cataracts, age-related macular degeneration, rheumatoid arthritis and a variety of neurodegenerative diseases (Hogg, 1998; Morrissey and O'Brien, 1998; Knight, 1998; Surai and Sparks, 2001). In animal production free radial generation and lipid peroxidation are responsible for the development of various diseases as well as for a decrease in animal productivity and product quality (Hurley and Doane, 1989; Weiss, 1998; Bottje and Wideman, 1995; McDowell, 2000; Surai and Dvorska, 2001).

Vitamin E is a major component of the antioxidant system and has received substantial attention in recent literature. However, less attention has been paid to the chemical reactions in which vitamin E exerts its antioxidant properties. It is well known that vitamin E (Toc-OH) effectively scavenges peroxyl radicals (ROO*) in the following reaction:

$$\mathbf{ROO^* + Toc\text{-}OH \longrightarrow ROOH + Toc\text{-}O^*}$$

As a result of this reaction a tocopheroxyl radical (Toc-O*) and a hydroperoxide (ROOH) are produced. The tocopheroxyl radical can be returned to the active form of tocopherol by recycling reactions with other antioxidants including ascorbic acid, glutathione, carotenoids, and ubiquinol, (for a review and references see Surai, 1999). The second product of the antioxidant action of vitamin E is a hydroperoxide. Hydroperoxides are toxic; and if not removed impair membrane structure and function (Gutteridge and Halliwell, 1990). Lipid hydroperoxides are unstable and in the presence of transition metal ions can decompose to produce new free radicals and cytotoxic aldehydes (Diplock, 1994):

$$ROOH + Fe^{2+} \longrightarrow RO^* + OH^- + Fe^{3+}$$
$$ROOH + Fe^{3+} \longrightarrow ROO^* + Fe^{2+} + H^+$$

These reactions account for much of the stimulation of lipid peroxidation by transition metal ions in biological systems (Halliwell and Gutteridge, 1999). Therefore hydroperoxides must be removed from the cell in the same way as H_2O_2, but catalase cannot react with these compounds. Only Se-dependent GSH-Px can convert these compounds into non-reactive products (Brigelius-Flohe, 1999):

$$ROOH + 2GSH \xrightarrow{\text{Se-GSH-Px}} ROH + GSSG + ROH$$

Therefore it appears that as the major antioxidant in the biological system, vitamin E, performs only half the job of removing free radicals and producing hydroperoxides. The second part of the process is dependent on the activity of Se-GSH-Px.

The importance of Se in animal nutrition lies in the fact that both first (detoxification of H_2O_2 formed by SOD action) and second (detoxification of hydroperoxides) levels of antioxidant defence in the cell rely on the activity of GSH-Px, which in turn depends on adequate Se status in the cell. Furthermore, even at very high levels of dietary vitamin E there is a need for Se (Surai, 2000). This is in agreement with data showing that high levels of dietary vitamin E do not replace cellular GSH-Px in protecting mice from acute oxidative stress (Cheng *et al.*, 1999). During Se deficiency lipid peroxidation is accelerated and damage to biological molecules can be lethal for the cell (Halliwell and Gutteridge, 1999). For example, if H_2O_2 or ROOH are not removed from the cell, they can damage molecules such as enzymes. Even more importantly, H_2O_2 can take part in the formation of other more active free radicals including the hydroxyradical (OH*), which is considered the most damaging radical in biological systems (Jaeschke, 1995).

Our observations (Surai, 1999; 2000; 2000a; Surai and Dvorska, 2001) indicate that a delicate antioxidant/prooxidant balance in the body is an important determinant of chicken health, embryonic development, sperm quality and probably productive and reproductive characteristics of poultry. There are different ways in which the antioxidant system can be altered or regulated. The most important regulation is the animal response to stress conditions by synthesising antioxidant enzymes, for example SOD and GSH-Px. However, this response will be effective only if cofactors such as Se for GSH-Px and Cu, Zn and Mn for SOD are available. Therefore, dietary Se is a crucial factor regulating GSH-Px activity and the efficiency of the antioxidant system.

Occurrence and feed sources

In nature Se exists in two chemical forms, organic and inorganic. Inorganic Se can be found in different minerals in the form of selenite, selenate and selenide as well as in the metallic form. In contrast, in forages, grains and oilseed meals Se is bonded to different amino acids including methionine and cysteine. Therefore, in nature animals receive Se mainly in the form of selenomethionine (SeMet; Combs and Combs, 1984). Plants absorb Se from the soil in the form of selenite or selenate and synthesise selenoaminoacids with SeMet representing about 50% of the Se in cereal grains (Olson and Palmer, 1976) and Se-methyl-selenomethionine, selenocysteine and Se-methyl-selenocysteine being the other seleno-compounds found in plants (Brody, 1994).

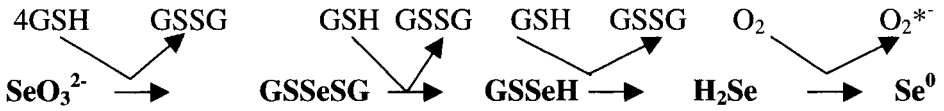
The Se concentration in soil varies significantly (Reilly, 1996); and its availability to plants depends on many factors. In the case of acidic soil pH or low soil aeration, Se can form insoluble complexes with iron hydroxide and become poorly available. Consequently, the Se content of animal feed ingredients also varies. As a result, dietary Se supplementation is an effective means to overcome Se deficiency and to maintain high productive and reproductive performance. The major Se supplements in use for the last 20 years are selenite and selenate-both inorganic forms of Se. The limitations of using inorganic Se are well known: toxicity, interactions with other minerals, low efficiency of transfer to milk, meat and eggs and inability to build and maintain Se reserves in the body. As a result a high proportion of the element consumed is simply excreted. Furthermore, a prooxidant effect of the selenite ion (Spallholz, 1997) is a great disadvantage. Thus, the use of sodium selenite in animal diets has recently been questioned (Pehrson, 1993). In contrast, SeMet itself is considered to possess antioxidant properties (Schrauzer, 2000). The development and commercialisation of organic Se, which contains >50% of total Se in the form of SeMet, provides a means of supplying animals with the same selenoaminoacids they could obtain from Se-adequate feed ingredients. This opens a new era in animal nutrition providing opportunities not only for improvement of animal health and productivity but also for production of Se-enriched meat, milk, eggs and other foods considered to be important steps in the improvement of human diets.

Prooxidant properties of selenite and possible antioxidant protection by SeMet

It is somewhat surprising that the most commonly used inorganic selenocompound, sodium selenite, is capable of promoting superoxide radical formation and oxidative stress through its reductive reaction with reduced glutathione. Generation of superoxide radicals by the reaction of selenite with reduced glutathione was first reported in 1988 (Garberg *et al.*, 1988; Kramer and Ames, 1988; Seko *et al.*, 1989). The important insight into the mechanisms of ROS formation came from the work of Yan and Spallholz (1993). In their experiment sodium selenite, sodium selenate, selenocystine and SeMet were tested for their abilities to generate superoxide radicals by the oxidation of glutathione and other thiols in the absence or presence of the human mammary tumour cell line HTB123/DU4475. The data suggested that a superoxide radical and H_2O_2 are produced from the reaction of selenite and selenocystine with glutathione. For example, in the absence of tumour cells, free radical generation (lucigenin-dependent chemiluminescence) was observed from the reaction of selenite with the thiols glutathione, 2-mercaptoethanol and L-cysteine, but not with SeMet. Superoxide dismutase, catalase, and GSH-Px all suppressed the observed chemiluminescence; but when these enzymes were heat inactivated they had little suppressive effect. The enhanced

ROS production by selenite and selenocystine in the presence of tumour cells was also suppressed by superoxide dismutase, catalase and GSH-Px.

Later the techniques used in those early experiments to detect ROS were questioned (Seko and Imura, 1997). However, a great deal of evidence analysed by Spallholz (1994) clearly showed that selenite Se is a prooxidant catalyst. Spallholz concluded that Se compounds are toxic owing to their prooxidant catalytic activity to produce superoxide ($O_2^{\cdot-}$), hydrogen peroxide, and very likely other cascading oxyradicals. The proposed scheme of superoxide radical generation was as follows (Seko *et al.*, 1989; Spallholz, 1997; Shen *et al.*, 2000):



Many Se compounds have been assessed in relation to their ability to produce superoxide *in vitro* (Spallholz, 1997) and the results are summarised in *Table 1*. Similarly, in their review of the prooxidant action of Se compounds Seko and Imura (1997) showed that it was possible to register superoxide radical and in some cases hydroxyl radical formation as a result of a selenite and glutathione reaction using different techniques including luminol dependent chemiluminescence, salicylate hydroxylation, decomposition of deoxyribose, single breakage of plasmid DNA and electron spin resonance. Free radical production was shown to be dependent on oxygen concentration and was ultimately responsible for Se toxicity (Seko and Imura, 1997). This was based on the results of several investigations indicating increased thiobarbituric acid reactive species (TBARS) production and other indexes of lipid peroxidation in cases of Se toxicity (Seko and Imura, 1997; Hoffman *et al.*, 1991; Csallany *et al.*, 1984). Based on the *in vitro* results and some *in vivo* findings, Seko and Imura suggested that Se compounds are able to generate ROS within tissues *in vivo*. In contrast, in most experiments SeMet was not able to produce ROS when added to an incubation medium in combination with reduced glutathione.

Table 1 Ability of selenium compounds to generate superoxide *in vitro.**

Superoxide produced *in vitro*	Superoxide not produced *in vitro*
Selenite	Selenomethionine
Selenium dioxide	Selenate
Selenocystine	Elemental selenium
Diselenodipropionate	Selenobetaine
Diphenyldiselenide	K-selenocyanate

*Adapted from Spallholz (1997).

It has been confirmed (Terada *et al.*, 1999) that selenite generates ROS and causes cellular damage in the presence of sulphhydryl compounds. When Se in the form of selenite, selenate or SeMet were added to total parenteral nutrition fluid and administered intravenously, selenite generated ROS in the presence of clinical concentrations of sulphhydryl compounds. This resulted in significant increases in the [^{3}H]-adenine and lactate dehydrogenase release rates from cells, a significant decrease in the amount of cellular protein, and enhancement of cellular damage compared with exposure to selenite alone.

The effects of selenite on DNA integrity, cell viability, and long-term proliferative potential of mouse leukaemic L1210 cells were examined by Lu *et al.* (1994). Selenite

treatment resulted in concentration-dependent increases in DNA single-strand breaks and double-strand breaks. A time-course experiment showed that DNA single-strand breaks preceded DNA double-strand breaks. Agarose gel electrophoresis of DNA extracted from selenite-treated cells displayed a nucleosomal fragmentation pattern that is characteristic of apoptotic cell death. Therefore, selenite treatment of a mouse mammary tumour cell line rapidly induced DNA damage and cell death (Lu *et al.*, 1995; 1995a).

In experiments with human colonic carcinoma cells, selenite (>5 µM) decreased cell growth, increased cell detachment and decreased intracellular levels of reduced glutathione (GSH), whereas >10 µM selenite induced cell differentiation and apoptosis (Stewart *et al.*, 1997). When primary cultures of human keratinocytes, melanocytes or the HaCaT cell line were preincubated with Se it has been shown that selenite was much more toxic to cells than SeMet (Raferty *et al.*, 1998). For example, 1 µM selenite killed approximately 25% of cells, and at a dose of 10 µM more than half the cells were destroyed. However, in the same experiment SeMet showed no toxicity at the doses used in the study. In an experiment with human promyelocytic leukaemia HL-60 cells, the dose-response data of apoptosis induced by selenite or selenodiglutathione were similar to those of cytotoxicity, implicating a relationship between the induction of apoptosis and cytotoxicity (Cho *et al.*, 1999). Therefore, superoxide radical formation and oxidative stress are related to the induction of apoptosis in selenite-exposed cancer cells (Stewart *et al.*, 1999; Shen *et al.*, 1999; Shen *et al.*, 2000). In cell culture, direct exposure of human umbilical vein endothelial cells to selenite induced cell death predominantly through apoptosis, decreased the gelatinolytic activities of matrix metalloproteinase-2 (Jiang *et al.*, 1999).

The experimental results of Stewart *et al.* (1999) suggest that selenite and selenocystamine generated 8-hydroxydeoxyguanosine DNA adducts, induced apoptosis and were found to be cytotoxic in mouse keratinocytes. On the other hand SeMet was not cytotoxic, did not generate 8-hydroxydeoxyguanosine adducts and did not induce cellular apoptosis at any of the Se concentrations studied. Therefore, in keratinocytes, apoptosis may be initiated by superoxide (O_2^{*-}) and oxidative free radicals that are generated by selenite and selenocystamine, but not by SeMet. Co-incubation of ascorbic acid or $CuSO_4$ 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 (Shen *et al.*, 2001).

Menter *et al.* (2000) showed that sodium selenite is a potent inducer of apoptosis in normal and cancerous prostate cells. At the same time SeMet selectively induces apoptosis in cancer but not primary cells of the human prostate. Similarly, Sundaram *et al.* (2000) showed that selenite had a significant inhibitory effect on growth of tumour cells but had little effect upon dermal fibroblasts that had been passaged numerous times. Se also induced mitochondrial damage and high rates of apoptosis in two brain tumour cell lines and in minimally passaged fibroblasts. These results showed clearly the damaging effect of selenite on cells and indicated that some types of cells after repeated passages can develop resistance to Se damage. Sodium selenite also exerted clear cytotoxic effects on a human hepatoma cell line. Shen *et al.* (1999) showed that Se-induced cell death occurs predominantly in the form of apoptosis. The involvement of glutathione in selenite-induced oxidative stress was further demonstrated by the concurrent decline of intracellular reduced glutathione and an increase in the oxidised glutathione content of Se-treated cells. Moreover, the finding that selenite-induced oxidative stress and apoptosis was significantly attenuated by superoxide dismutase, catalase and deferoxamine provides additional evidence to suggest that Se-induced oxidative stress mediates the induction of apoptosis. Recently, Shen *et al.* (2001) provided convincing evidence that the intracellular O_2^- formed through the reaction of selenite with GSH is a potent proapoptotic agent and mainly acts on mitochondria to trigger the apoptotic signalling pathway.

The prooxidant effect is probably tissue-dependent reflecting the antioxidant composition and concentrations in each tissue studied. For example, the effect of sodium selenite (0.05, 0.1, and 0.2 mg/kg body weight) on the thiobarbituric acid reactive substances and groups in the striatum and thalamus of a male Wistar rat was studied after 7 days of treatment (Zia and Islam, 2000). The content of TBARS was elevated dose dependently in the striatum, but its level was depleted significantly with the 0.1 mg/kg dose of sodium selenite in the thalamus. In general, Se toxicity occurs as a result of increased thiol oxidation, redox cycling and superoxide generation in a dose dependent manner (Stewart *et al.*, 1999). Vitamin E- and Se-deficient rats given selenite produced 15 times as much ethane as did controls (Dougherty and Hoekstra, 1982). It was concluded that the increased vulnerability of vitamin E- and Se-deficient rats to acute selenite toxicity might involve peroxidation *in vivo*.

Se prooxidant action and cytotoxicity is an important subject in relation to the anticarcinogenic action of this trace element. In fact, it has been demonstrated that cancer cells had higher sensitivity to Se cytotoxicity in comparison with normal cells (Stewart *et al.*, 1997; Medina and Oborn, 1981; Fico *et al.*, 1986). This difference in sensitivity to Se can reflect differences in glutathione concentration in different cells. For example, Shen *et al.* (2000) demonstrated that GSH has a dual role in Se cytotoxicity acting as a prooxidant to induce oxidative stress or as an antioxidant to protect against Se-induced oxidative stress.

It is interesting to note that in most of the experiments reported above ROS generation and cellular damage were not observed after simultaneous administration of various concentrations of selenate or SeMet with sulphhydryl compounds (Terada *et al.*, 1999). SeMet is a relatively non-toxic, non-catalytic and non-redoxing Se compound exhibiting low toxicity and not producing superoxide (Stewart *et al.*, 1999). In fact, when Se as the selenite, SeMet, ebsleben (2-phenyl-1,2-benzisoselenazol-3(2H)-one) 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 H_2O_2 caused an inhibition in peroxyl radical formation in a manner dependent on SeMet concentration (Sun *et al.*, 1997). Furthermore, SeMet is considered as a powerful antioxidant protecting against damaging effects of peroxynitrite. For example, it protected human fibroblast lysates from the toxic effect of peroxynitrite (Sies *et al.*, 1998). SeMet also protected dihydrorhodamine 123 from oxidation and 4-hydroxyphenylacetate from nitration caused by peroxynitrite while sodium selenite exhibited no effect (Briviba *et al.*, 1996), and protected DNA from single-strand breaks induced by peroxynitrite (Roussyn *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 utilising two equivalents of thiol has been described (Assmann *et al.*, 1998). Radioprotective and UV-protective properties of SeMet (Schrauzer, 2000) may also be associated with its antioxidant properties.

Se has also been shown to have a protective effect on gap junctional communications diminished by peroxynitrite (Sharov *et al.*, 1999). The antioxidant properties of SeMet were also demonstrated in a model system based on the olive oil oxidation process at 303K and 333K (Zalejska-Fiolka, 2000).

From analysis of the antioxidant/prooxidant properties of Se it is possible to draw the following general conclusions:

- Selenite is potentially highly toxic due to reactions with glutathione and possibly other sulphhydryl compounds present in biological systems that ultimately produce ROS. In contrast SeMet does not participate in prooxidant reactions and in some cases possesses antioxidant properties.

- The prooxidant properties of selenite and other Se compounds are an important finding to aid in the explanation of some of the anticancer effects of Se. However, in a physiological context prooxidation effects can be detrimental. For example, increased meat drip loss during storage (Mahan, 1999; Edens, 2001) could be a result of the prooxidant action of selenite inclusion in food animal diets.
- The prooxidant actions of selenite should be studied in relation to possible oxidative reactions in the digestive tract of animals, resulting in decreased nutrient assimilation and development of various diseases. Indeed, feed contains a range of antioxidants (tocopherols, tocotrienols, carotenoids, flavonoids etc.) as well as prooxidants (Fe, Cu, hydroperoxides etc.). In such conditions the oxidising potential of Se compounds can have a substantial effect on lipid peroxidation. At present, the oxidation and redox control of pathophysiological processes in the gastrointestinal tract is not well understood (Aw, 1999). In general, enterocytes are characterised by comparatively high turnover. For example, in rats enterocyte turn over occurs within 48-72 h (Miller *et al.*, 1977). The intestinal mucosa is constantly challenged by diet-derived oxidants, toxic compounds and free radicals and toxic products of their metabolism (Ames, 1983; Aw, 1999). In such conditions antioxidant protective mechanisms in the intestine are the first line of defence against all those toxic elements. Therefore, in order to maintain cellular integrity and tissue homeostasis, the intestine has a range of defence mechanisms (Aw, 1999), many of which are Se-dependent. For example, gastrointestinal GSH-Px is the major protection against oxidative stress in the intestine. Tocopherols, tocotrienols, carotenoids, ascorbic acid and glutathione, which are released from the feed during digestion, are also of great importance. There are other less studied nutrients with antioxidant properties such as flavonoids, which can also contribute to antioxidant defence. However, the most important defence in the intestine is provided by up-regulation of antioxidant enzymes as well as by signalling mechanisms responsible for cell death by apoptosis to dispose of injured or spent enterocytes (Aw, 1999). Indeed, intestinal glutathione is considered responsible for maintenance of cellular redox balance and respective gene regulation (Wang *et al.*, 2000; Aw, 1998).
- The pro-oxidant properties of selenite are proven, but the molecular mechanisms of this effect need further investigation. However, using organic Se in the form of SeMet could be a valuable alternative to avoid the detrimental consequences of the pro-oxidant properties of selenite.

Selenium deficiency

Se deficiency in the chicken, especially in combination with low vitamin E supply, is responsible for the development of a range of diseases including exudative diathesis (Nouguchi *et al.*, 1973; Barthlomew *et al.*, 1998), nutritional encephalomalacia (Century and Hurwitt, 1964; Combs and Hady, 1991) and nutritional pancreatic atrophy (Thompson and Scott, 1969; 1970; Cantor *et al.*, 1975). Nutritional pancreatic atrophy (NPA) is considered to be the only clearly defined Se deficiency syndrome uncomplicated by deficiencies of other antioxidants (Combs, 1994). Se deficiency uncomplicated by vitamin E deficiency was produced in chickens by an amino acid diet complete in all known nutrients except Se (Noguchi *et al.*, 1973; Gries and Scott, 1972).

It seems likely that in the aetiology of Se-deficiency related diseases, lipid peroxidation is a major factor (Fraga *et al.*, 1987). For example, nutritional pancreatic atrophy in chicks may be overcome by feeding vitamin E at 15-20 times the levels normally regarded as nutritionally adequate (Whitacre *et al.*, 1987). Se supplementation can also decrease the incidence of nutritional muscular dystrophy in the chick (Jonsson, 1993).

Se deficiency is associated with impaired immunocompetence, reduced egg production and increased embryonic mortality (Combs and Combs, 1984). Hatchability of eggs was depressed by a low-Se diet, further depressed by peroxidised fat and was restored to normal by supplementation with Se and vitamin E (Combs and Scott, 1977). Similarly to chickens, Se is required in breeder turkey diets for optimum hatchability and viability of offspring (Cantor *et al.*, 1978). Fertility and hatchability were low on the basal (low Se) diet and were corrected partly by vitamin E and completely by Se (Latshaw and Osman, 1974). Egg production and fertility were maintained at about 77% and 92%, respectively, by the Se diet and fell to about 56% and almost zero with the basal low Se diet (Cantor and Scott, 1974). Egg production was only 69% in Se-deficient birds against 81% in the controls. Eggs from hens fed very low levels of Se were more often infertile (12.6%), there were more dead embryos (29%) and lower hatchability of fertile eggs (15%). Mean respective values for the controls were 4.1, 2.9 and 91% (Latshaw *et al.*, 1977). Day-old chicks from hens given the 0.05 and 0.1 mg/kg Se-supplemented diets were significantly heavier than those from hens given no Se. It is also interesting to note that exudative diathesis was observed at hatching, indicating that the deficiency lesions had developed during the embryonic period (delete Hassan *et al.*, 1990).

Recent understanding of the roles of selenoproteins as elements of antioxidant systems could help to explain some clinical signs of diseases. Furthermore, a consideration of Se as an integral part of the antioxidant system also helps to explain complex relationships between individual antioxidants in the biological system. For example, recent results show that liver cells can boost endogenous ubiquinone-dependent protective mechanisms in response to deficiency in vitamin E and Se (Navarro *et al.*, 1999). Therefore in the absence of vitamin E and Se, enhancement of ubiquinone-dependent reductase systems can protect the membrane against peroxidation (Navarro *et al.*, 1998). Similarly, Se participation in the regulation of redox status of the cell can be crucial for explaining some signs of its deficiency. In this respect, the selenoenzyme thioredoxin reductase, which is involved in regulation of many metabolic reactions in the cell, could be the major target for future research.

Selenium toxicity and selenosis

Se is toxic to poultry when used in high doses. However it is necessary to stress that Se toxicity can usually only be seen when its dose exceeds the physiological requirement at least 10-fold. Data on this topic are sometimes contradictory, but Se doses lower than 3-5 mg/kg feed are usually not associated with toxicity.

When White Leghorn chickens were fed a basal diet containing 0.30 mg Se/kg supplemented with 0, 0.1, 0.5, 1.0, 3.0 and 6.0 mg Se/kg in the form of SeMet for 18 weeks, no toxic effects were observed, even at the highest intake of Se (Moksnes, 1983). From 40 days of age two groups of chickens were given cystine selenate at 2 mg/kg of drinking water for 250 days. A control group received no added Se. There was no effect of Se on growth or egg production, however anaemia and stiffness of the tibiotarsal joints were observed (Soffietti *et al.*, 1983). In a trial with 40 Leghorn hens and five cockerels dietary Se of 0.14 to 0.85 mg/kg dry matter (DM) had no adverse effect on the parent birds or on hatching of eggs (Kaantee *et al.*, 1982). When Se as sodium selenate was added to the feed from 0.1 up to 9 mg /kg, hatchability of fertile eggs was significantly decreased only by 5 mg/kg Se or higher, egg weight by 7 mg/kg or higher and egg production was decreased only by 9 mg/kg (Ort and Latshaw, 1978). Hens and chickens given 8 to 35 mg sodium selenite/kg bodyweight through the diet or into the crop (Akulov *et al.*, 1972) showed signs of Se toxicity after 15 to 30 minutes of crop administration. In chickens,

signs appeared 3 to 4 days after intake of Se in the feed. Other groups of hens were given dietary Se at 0.8, 2.4, 4.0, 6.4 or 9.0 mg sodium selenite Se/kg bodyweight for 40 days. Egg production was unaffected by the lowest intake, but at intakes exceeding 0.8 mg/kg, it dropped from 64 to 8.7% and at the highest intake, 6.4 or 9.0 mg/kg, egg laying ceased (Akulov *et al.*, 1972). Todorovic *et al.* (1999) fed day-old unsexed Hybro chickens basal diets supplemented with 0, 2, 5, 10, 20 and 30 mg Se/kg as sodium selenite for six weeks. The lowest level at which dietary Se caused reduction in daily gain was 5 mg/kg. Diets supplemented with 10, 15 and 20 mg Se/kg produced 24.5, 62.7 and 96.6% reductions in daily gain, respectively. Feeding diets with 15, 20 and 30 mg Se/kg caused 26.7, 60 and 80% mortality, respectively (Todorovic *et al.*, 1999).

The reproductive effects of Se were studied in American kestrels (*Falco sparverius*) fed diets containing 6 or 12 mg Se/kg DM as SeMet for 11 weeks. Fertility was reduced in the 12 mg/kg group, but egg production, hatchability and incidence of embryonic malformations were unaffected (Santolo *et al.*, 1999). Chronic Se toxicosis was induced in one-year-old male mallard ducks by feeding Se as SeMet in amounts of 0, 10, 20, 40, and 80 ppm for 16 weeks. All mallards in the 80 ppm group, three in the 40 ppm group, and one in the 20 ppm group died (Green and Albers, 1977). No significant histological lesions were detected in euthanised mallards fed on the diet with 0, 10 or 20 ppm SeMet (Green and Albers, 1977).

The oral LD_{50} of Se (in the form of sodium-selenite) for chickens was reported to be 9.7 mg/kg body mass (Salyi *et al.*, 1993). In another experiment the LD_{50} for chickens was calculated as 24.6 mg Se/kg body weight (Tishkov and Voitov, 1989). The authors also found species-specific differences in susceptibility to Se toxicity indicating LD_{50} for turkey poults and ducks to be 13.5 and 64 mg/kg body weight, respectively. The minimum toxic dose of sodium selenite by the oral route was 0.9 mg/kg body weight for turkey poults, 1.7 for broiler chicks and 9.4 for ducks (Tishkov and Voitov, 1989). In laying hens the LD_{50} was 33.4 mg/kg body weight and the maximum tolerated dose was 15 mg/kg body weight (Akulov *et al.*, 1972).

An *in ovo* treatment (0.15, 0.30, 0.45 and 0.60 ppm of added Se per embryo) depressed embryo wet weights at 3 and 4 days of incubation. Embryonic mortality resulting from the above Se treatments was 16.2, 15.1, 28.2 and 29%, respectively (control mortality was 8.2%), and 99% of these embryos did not develop beyond the six-day stage (Fitzsimmons and Phalaraksh, 1978). Gross lesions of Se toxicity in chick embryos included webbed, fused and curled toes and cracked, crooked and shortened beaks. Histopathological studies showed that Se injected as selenite caused dissociation of hepatic cells, particularly around the central veins. The glomeruli of affected kidneys were enlarged, and in each glomerulus the lumen of the capillary tuft was dilated. Epithelial cells of the proximal convoluted tubules were detached from the basement membranes (Sukra *et al.*, 1976).

Growth depression and reduced egg production, anaemia and stiffness of the tibiotarsal joints are characteristic of Se overdose (Soffietti *et al.*, 1983). Se-fed chicks had increased relative liver and heart weights (Khan *et al.*, 1993). When chickens were given toxic doses of organic Se (8-13 ppm in Se-enriched corn), the predominant pathological changes were characterised by local necrosis in the liver, myocardial degeneration and convoluted tubule necrosis in the kidneys (Qi *et al.*, 1992). In birds that died as a result of selenosis the following alterations were observed: hepatic degeneration with increasing severity; diffuse tubulo-nephrosis followed by necrosis of the tubular epithelium; myocardial and skeletal myodegeneration, as well as damage to the bursa of Fabricius and cerebellar oedema (Salyi *et al.*, 1993). Dyspnoea, watery diarrhoea, weakness and somnolence were observed within a short time when Se poisoning was induced experimentally (Salyi *et al.*, 1993).

Histological lesions in mallards that died of selenosis were hepatocellular vacuolar degeneration progressing to centrolobular and panlobular necrosis, nephrosis, apoptosis of

pancreatic exocrine cells, hypermaturity and avascularity of contour feathers of the head with atrophy of feather follicles, lymphocytic necrosis and atrophy of lymphoid organs (spleen, gut-associated lymphoid tissue, and lumbar lymph nodes), and severe atrophy and degeneration of fat (Green and Albers, 1997). In the same experiment histological lesions in surviving mallards included atrophy of lymphoid tissue, hyalinogranular swelling of hepatocytes, atrophy of seminiferous tubules and senescence of feathers (Green and Albers, 1997).

The molecular mechanisms of Se toxicity are not well defined. For example, it has been suggested that substitution of Se for the sulphur in keratin could result in weakened physical protein structure and failure of keratinised tissue (for a review see Raisbeck, 2000). However, considerable evidence has accumulated suggesting oxidative stress as the main molecular mechanism of selenosis (Raisbeck, 2000). Indeed a reaction between selenite and glutathione with production of free radicals can explain the prooxidant properties of selenite. The primary targets of acute Se toxicity in food animal species are the cardiovascular, gastrointestinal and haematopoietic systems (Raisbeck, 2000). However, organic Se in high doses is also toxic, but SeMet does not produce free radicals when reacting with glutathione. Therefore molecular mechanisms of Se toxicosis need further investigation.

Conclusions

Se plays an important role in the regulation of various metabolic processes in the body, being an integral part of selenoproteins. Organic Se in the form of selenomethionine is a predominant form of this element in feed ingredients. Therefore the digestive system of animals, including chickens, has adapted to this form of the element during evolution. In this regard selenite (a common form of Se used in diets) is not found naturally and as a result is less effective in terms of assimilation from the feed and building Se reserves in the body. Se deficiency and toxicity are rare events in poultry production. However, the precise Se requirement of various poultry species in commercial conditions needs further elucidation.

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