The importance of antioxidants in poultry

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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. Among many dietary factors, antioxidants have a special place being major players in the battle for animal survival, maintenance of animal health, productive and reproductive performance. This is largely because of the detrimental effects of free radicals and toxic products of their metabolism on various metabolic processes.

Do not free radicals

Free radicals are atoms, molecules or any compounds containing one or more unpaired electrons. Most biologically-relevant free radicals are derived from oxygen and nitrogen. Both these elements are important for animal life, but in some circumstances they can be converted (deliberately or by chance) into free radical molecules. Free radicals are highly unstable, very reactive and are capable of damaging molecules such as DNA, proteins, lipids or carbohydrates. Damage to DNA is associated with mutations, translation errors, and disruption of protein synthesis. In some cases damage to DNA leads to cancer. Damage to proteins causes modifications in ion transport and receptor functions, as well as altered enzymatic activities. Polyunsaturated fatty acid oxidation alters membrane composition, structure and properties (fluidity, permeability, etc) and activity of membrane-bound enzymes. The damage to biological molecules ultimately compromises growth, development, immunocompetence and reproduction.

Cells are under constant attack by free radicals, many of which are formed as a natural consequence of normal metabolic activity and as part of the immune system's strategy for destroying invading microorganisms (Table 1). For example, under normal physiological conditions about 3-5% of the oxygen taken up by the cell undergoes univalent reduction leading to the formation of free radicals (Singal et al., 1998). About 10^{12} O_2 molecules processed by each rat cell daily and the leakage of partially reduced oxygen molecules is about 2%, yielding about 2 x10^{10} molecules of reactive oxygen species (ROS) per cell per day (Chance et al., 1979). Furthermore Helbock et al. (1998) have shown that the DNA in each rat cell was 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 increased with age. An old rat had accumulated about 66,000 oxidative DNA lesions per cell (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 to form superoxide radical and took into account that an adult at rest utilises 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% form 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 amount would be substantially increased. In addition to free radicals formed as a natural consequence of oxygen metabolism, immune cells produce free radicals as a means to destroy pathogens (Kettle and Winterbourn, 1997). These calculations show that free radical production in the body is substantial and underscore the fact that thousands of biological molecules can be easily damaged if not protected.

Natural antioxidants

For the majority of organisms on earth, life without oxygen is impossible, animals, plants and many microorganisms relying on oxygen for the efficient production of energy. However, the high oxygen concentration in the atmosphere is potentially toxic for living organisms. During evolution specific antioxidant evolved as protective mechanisms against the free radicals continually being produced. These mechanisms allowed survival in an atmosphere where oxygen concentration was rising.

In nature there are thousands of compounds possessing antioxidant properties. There are both 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 are delivered with food/feed (vitamin E, carotenoids, Se etc.). The antioxidant enzymes that are synthesised in the body require metal co-factors. For example selenium in the form of selenocysteine is at the active site of several families of enzymes such as the glutathione peroxidases (GSH-Px) and thioredoxin reductase (TR). Zinc, copper and manganese are integral parts of another antioxidant enzyme family called superoxide dismutases (SOD); and iron is an essential part of the antioxidant enzyme called catalase. Only when these metals are delivered with the diet in sufficient amounts can the body synthesise the antioxidant enzymes. In contrast, deficiency of those elements causes oxidative stress and allows damage to biological molecules and membranes.

It is important to realize that all antioxidants in the body function in concert to provide antioxidant defence. The role of each member of the antioxidant ‘team’ is well defined. For example, selenium is part of several different selenoproteins that regulate antioxidant defence in different tissues and in different ways. It is primarily, but not exclusively intracellular. Vitamin E provides antioxidant defence in lipids, specifically biological membranes. The function of each is important to the effectiveness of the other antioxidants. For example, vitamin C ‘recycles’ vitamin E from oxidized form to become active again, while glutathione does the same for vitamin C. If the diet is balanced and sufficiently provided with dietary antioxidants and antioxidant nutrients, then even low doses of such antioxidants as vitamin E are effective. On the other hand, under conditions of oxidative stress where free radical production dramatic increases, then without external help it is difficult to prevent damage to major organs and systems. This external help takes the form of increased dietary supplementation of natural antioxidants, especially vitamin E and selenium. For the nutritionist or feed formulator the challenge is to understand when and how much external ‘help’ is needed and economically justified. The list of stressors in poultry production is shown below (Surai, 2002a):

- *Time* between when the egg is laid and its cooling down for storage. Eggs should be collected more frequently on warm days. In such conditions free radical damage to lipids and proteins can occur and additional antioxidant protection would be beneficial.

- *Egg storage before incubation* could be associated with lipid peroxidation within the egg membranes, which contain high levels of PUFAs. Increased Se concentration in combination with other antioxidants (vitamin E and carotenoids) could be an effective means to prevent damaging effects of free radicals produced within the egg.

- *Temperature, humidity and carbon dioxide concentration* fluctuations during incubation. Fluctuation can also affect embryonic development, oxidation and phosphorylation in embryonic tissues leading to free radical production. For example, high carbon dioxide concentrations during incubation can jeopardise embryo liveability.

- *Day 19 of embryonic development* is an important point when risk of lipid peroxidation is very high. At this stage chick tissues are characterised by comparatively high levels of PUFAs. At the same time, concentrations of natural antioxidants (vitamin E and carotenoids) have not reached maximum. At this stage of development pipping occurs; and oxygen availability to tissues increases. Low antioxidant status in combination with high temperature, humidity, and PUFAs could increase susceptibility to lipid peroxidation.

- *Hatching time* is an environmental stress for the chick. At this point natural antioxidant concentrations have reached maximum, but high levels of lipid unsaturation in tissues, decreasing concentrations of ascorbic acid (can limit vitamin E recycling) and high temperature and humidity increase risk of lipid peroxidation.

- *Delay in collecting chicks from incubator*. Since not all chicks are hatched at the same time because of heterogeneity of the starting material (eggs from older breeders hatch earlier than those from young flocks and chicks from smaller eggs hatch earlier than those from large eggs), some would be in the incubator for 2-12 hrs longer than others. This puts pressure on antioxidant defence capacity. Furthermore, any delay in food and/or water intake after hatching usually negatively affects a number of performance parameters and a delay occurs in the maturation of the enzymatic systems that control metabolism and free radical production and protection against them.

- *Transportation from hatchery to farm* is another source of stress. For breeding companies where chicken transportation could involve several thousand miles, a very high degree of stress would be associated with temperature fluctuation and dehydration.

- *Suboptimal temperatures in the poultry house.* Cold tolerance as well as feather cover is influenced by thyroid hormone activity, which is Se-dependent through the selenoenzyme iodothyronine deiodinase.

- *High levels of ammonia and CO_2 in the poultry house as a result of inadequate ventilation.* This could substantially decrease antioxidant system efficiency.

- *Disease challenge.* Phagocytic immune cells themselves produce free radicals in the process of killing internalised pathogens. Without adequate antioxidant nutrient reserves, cellular machinery can be damaged by the free radicals thereby reducing the effectiveness of the immune cell. In addition, Se is considered to have a specific role in immune system regulation, which could be independent of its antioxidant functions.

- *Vaccination* is also a substantial stress; and in some cases using vitamin E, for example, as a vaccine adjuvant can help improve vaccination efficiency.

- *Induced moulting with feed withdrawal* is an important stress condition when decreased efficiency of heterophil function increases bird susceptibility to various infections.

- *Mycotoxins* can substantially decrease antioxidant assimilation from the feed and increase their requirement to prevent damaging effects of free radicals produced as a result of mycotoxin exposure.

- *Heavy metals and other toxicants (dioxine, pesticides, fungicides, herbicides, etc.) in the feed* can also cause oxidative stress, decreasing immunocompetence, productive and reproductive performance and increasing requirements for antioxidants.

- *Oxidized fat in the diet* can cause oxidative stress in the intestine increasing antioxidant nutrient requirements. When a chicken diet includes spent fat after its high temperature treatment, the fat usually contains peroxides and hydroperoxides that can contribute substantially to oxidative stress. It is necessary to evaluate benefits vs disadvantages of using such fat sources.

- *Extensive preventive medication (coccidiostats or other veterinary drugs in the diet)* can decrease antioxidant assimilation from the diet or increase their requirement to deal with generated stress conditions. For example, monensin can stimulate lipid peroxidation in the chicken liver. Similarly, oral furazolidone treatment of chickens was associated with a decreased vitamin E concentration and increased lipid peroxidation in their liver.

- *Vitamin A excess* in the diet is shown to cause an oxidative stress decreasing vitamin E and carotenoid concentrations in tissues and increasing tissue susceptibility to lipid peroxidation.

The list of potential stresses can vary from one poultry farm to another, but overproduction of free radicals and the critical need for antioxidant protection are common factors.

Let's consider major steps of antioxidant defence to help a nutritionist to make a decision. Three major levels of antioxidant defence are shown in Figure 1.

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

Since first level of antioxidant defence is not sufficient to completely prevent free radical formation and lipid peroxidation, the second level of antioxidant defence is responsible for prevention and restriction of chain free radical reactions. They do this job

mainly by scavenging free radicals and converting them into less reactive compounds. For example, when vitamin E reacts with peroxyl radical, hydroperoxide is produced and vitamin E is oxidised:

Vit. E + ROO* = ROOH (hydroperoxide) + Vit.E-oxidized

In such reactions antioxidants are oxidized and can loose their activity. Due to recycling reactions the oxidized antioxidants can be regenerated back to active form. This explains why it is so difficult to produce vitamin E deficiency in adult animals. Surprisely 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) could generate a range of powerful free radicals. The main enzyme responsible for detoxification of the hydroperoxide is Se-GSH-Px. Vitamin E and Se are working in a tandem and even very high doses of vitamin E in the diet cannot replace Se which is needed (in the form of GSH-Px) to complete the second part of antioxidant defence as mentioned above. Therefore, Se-dependent GSH-Px is responsible for detoxification of this hydroperoxide and Se as an integral part of this enzyme belongs to the first and second levels of antioxidant defences.

Even the second level of antioxidant defence is not able to prevent damages to biological molecules and some lipids, proteins, carbohydrates 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.

Natural antioxidants in feed ingredients

It has been suggested that antioxidant/prooxidant balance in the body is responsible for maintaining human and animal health, productive and reproductive performances of farm animals. In general, an excess of free radicals, or lack of antioxidant protection, can shift this balance producing oxidative stress. It is well recognised that oxidative stress plays a major role in many degenerative pathologies and free radical formation is considered as a pathobiochemical mechanism involved in the initiation or progression phase of various diseases. In fact, it is widely believed that most of human and probably animal diseases at different stages of their development or progression are associated with free radical production and metabolism.

The antioxidant-prooxidant balance can be adversely modulated by sub-optimal diets and nutrient intakes or positively affected by dietary supplementation. Therefore, feed components can modulate maintenance of this balance and may thereby influence the rate of ageing as well as disease resistance of the human and animals. Thus, the most important step in balancing oxidative damage and antioxidant defence in the animal body would be to enhance the antioxidant capacity by optimising the dietary intake of antioxidants.

Animal diet contains a range of different compounds that possess antioxidant activities. Let's briefly consider main dietary antioxidants (Surai and Dvorska, 2002b).

Vitamin E

- main chain-breaking antioxidant in biological systems

- is located in biological membranes and lipid droplets
- absorbed in the small intestine with various efficiency depending on the diet composition, level of supplementation, age, sex and other individual characteristics of animals
- accumulated to some extent in the liver and adipose, however, this deposition is not sufficient to maintain physiological requirement for a long time
- crude plant oils are richest source of vitamin E, however, oil refining decreases vitamin E concentration dramatically.
- not stable and easily oxidized. Commercial preparations are usually contain esterified forms of the vitamin (e.g tocopheryl acetate) which is comparatively stable during storage but does not possess antioxidant activity itself, only after digestion in the intestine it is converted to active alpha-tocopherol
- not toxic and even very high doses are not associated with hypervitaminosis
- comparatively expensive
- recent data showed that increased vitamin E supplementations beneficial for animals in stress conditions
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Carotenoids

- natural pigments, responsible for yellow, orange and sometimes red pigmentation in plants, insects, birds and marine animals
- possess antioxidant activity
- have some health promoting properties, including immune system modulation
- there is no established requirements for animals
- some plant-derived feed ingredients are rich in carotenoids, but animal–derived feed ingredients are poor sources of carotenoids
- not stable and oxidized during food storage
- reserves in the body are limited

Vitamin C

- universal water-soluble antioxidant
- working in a close relationship with vitamin E, recycling it from oxidized form
- important anti-stressor agent
- synthesised in farm animals
- in stress conditions dietary supplementation is beneficial
- easily oxidized

Glutathione

- cellular antioxidant
- synthesised in animals
- intracellular redox status sensor
- important in stress conditions
- dietary supplementation is not proven to be effective

Selenium

- trace element
- essential part of a range of selenoproteins, including glutathione peroxidase (GSH-Px), thioredoxin reductase (TrxR), iodothironine deiodinase (ID) and some others
- food ingredients contain variable concentrations of Se, but most of them are deficient in this element

- physiological requirement is low, but if not met, antioxidant system is compromised with detrimental consequences for animal health
- in high doses is toxic
- there are two major sources of Se for animals: a natural source in the form of selenomethionine and inorganic selenium in the form of selenite or selenate
- it is proven that organic selenium has a range of advantages for animals

Zinc

It is the second most abundant trace element in mammals and is a component of over 300 enzymes and taking part in

- antioxidant defence as an integral part of SOD; hormone secretion; keratin generation and epithelial tissue integrity; nucleic acid synthesis; protein synthesis; sexual development and spermatogenesis; immune function

Organic Zn is characterised by improved availability in comparison to inorganic sources and is considered to be beneficial for animal health.

Copper

It is an essential component of a range of physiologically important metalloenzymes and taking part in:

- antioxidant defence as an integral part of SOD; cellular respiration; cardiac function; bone formation; carbohydrate and lipid metabolism; immune function; connective tissue development; tissue keratinization; myelination of the spinal cord

Inorganic copper has a strong prooxidant effect and if not bound to proteins could stimulate lipid peroxidation in feed or even more importantly in the intestinal tract. Organic copper does not possess prooxidant properties and can improve Cupper status of animals

Iron

It has a vital role in many biochemical reactions taking part in:

- antioxidant defence as an essential component of catalase; energy and protein metabolism; heme respiratory carrier; oxidation/reduction reactions; electron transport system

Iron is a very strong prooxidant and if not bound to proteins can stimulate lipid peroxidation. This is especially relevant to digestive tract where lipid peroxidation can be stimulated which can cause enterocyte damages and decreased absorption of various nutrients especially antioxidants. If iron is included in premix in inorganic form it can stimulate vitamin oxidation during storage. Therefore organic iron could be a solution to avoid those problems and improve iron status of animal.

Manganese

It is plays an important role in body metabolism as an essential part of a range of enzymes taking part in

- antioxidant protection as an integral part of SOD; bone growth and egg shell formation; carbohydrate and lipid metabolism; immune and nervous function; reproduction

Similar to other organic minerals organic manganese seems to be better assimilated from the diet

Let’s consider two major applications of natural antioxidants in relation to animal production, in particular their role in reproduction 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 animal spermatozoa should be characterised by high motility and acrosome integrity. Furthermore to be motile the spermatozoa should have intact mitochondria (energy-producing stations in the cell) and high membrane flexibility and fluidity. To maintain those membrane properties there is a need for high level of polyunsaturated fatty acids (Surai et al., 2003). In fact spermatozoa from all animal species are characterised by extremely high proportions of those fatty acids and as a result they become very vulnerable to oxidative stress due to overproduction of free radicals. To deal with those dangerous conditions the antioxidant system of the spermatozoa includes fat-soluble and water-soluble chain-breaking antioxidants as well as antioxidant enzymes.

Understanding of involvement of selenium in maintenance of semen quality came from data on selenoproteins. In particular, there are several selenoproteins, which are found in spermatozoa. For example, glutathione peroxidases (GSH-Px), a family of 5 important antioxidant enzymes, are responsible for prevention of damaging effects of free radicals and toxic products of their metabolism on spermatozoa. The last (5^{th}) member of this family of selenoproteins, so called sperm nuclear GSH-Px was identified only two years ago. It seems likely that thioredoxin reductase (TR) is also involved in antioxidant defence in spermatozoa, but there are no data available at present to confirm this. Furthermore, a specific sperm capsular selenoprotein is located in the midpiece of spermatozoa. Recently it was identified as phospholipid hydroperoxide glutathione peroxidase, a form of Se-dependent GSH-Px. Since mitochondria are the main source of free radicals in the spermatozoa and they are located in midpiece, antioxidant protection there is a crucial factor for sperm motility and fertilizing ability. For example, Se-deficiency caused various sperm abnormality in this region (Surai, 2002) resulting in decreased fertilising ability and organic selenium dietary supplementation is more efficient in improving semen morphology than selenite (Edens, 2002). Experiments with Sel-Plex in the cockerel diet proved the point: organic selenium also increases duration of fertility (Agate et al., 2000).

Natural antioxidants and chicken embryonic development

Chick embryo tissues contain a high proportion of highly polyunsaturated fatty acids in the lipid fraction and therefore need antioxidant defence. Tissues of newly hatched chicks express a range of antioxidant defences including natural antioxidants (vitamin E, carotenoids, glutathione, ascorbic acid) and antioxidant enzymes (superoxide dismutase, glutathione peroxidase and catalase) as well as antioxidant enzyme cofactors (Se, Zn, Mn and Fe) (Surai, 2002). Of these, vitamin E, carotenoids and metals, including Se, are delivered from the maternal diet via the egg and the others are synthesised in the tissues.

There are tissue-specific features in antioxidant composition. For example, the highest level of vitamin E was found in the liver and lowest in the brain. Carotenoids also were concentrated in the liver. However, brain was substantially enriched in ascorbic acid; and it was suggested that in the brain an effective recycling of vitamin E by ascorbic acid could maintain effective antioxidant protection even with low level of vitamin E. Highest Se-GSH-Px was observed in the liver and kidney, but lowest in the brain. In newly hatched chicks Se concentrations in tissues can be placed in the following order: liver>kidney>lung> heart>brain>muscle. Therefore, it has been suggested that brain lipid composition and antioxidant concentrations predispose this tissue to be most vulnerable to

lipid peroxidation. It might well be that these features of the brain are involved in nutritional encephalomalacia development in the case of low vitamin E and Se supplementation.

It is necessary to underline that maternal diet composition is a major determinant of antioxidant system development during embryogenesis and in early postnatal development. Vitamin E, carotenoids and selenium are transferred from feed into egg yolk and further to embryonic tissues. Our observations indicate that an increased antioxidant supplementation of the maternal diet can substantially increase their concentrations in developing chick tissues and significantly decreases susceptibility to lipid peroxidation. There is tissue specificity in Se transfer from egg to the embryo. For example, in contrast to the liver, there was only a trend (non-significant) toward higher Se accumulation in the brain of chickens hatched from the egg enriched in Se. It is important to mention that there was a positive effect of Se supplementation of the maternal diet on the levels of vitamin E in the liver, brain and blood plasma of day old chicks. A positive effect of Se supplementation of the maternal diet was seen at day 5 and 10 post-hatch when vitamin E concentrations in the liver and plasma were significantly elevated compared to controls. The mechanisms for this sparing is not clear, but could be related to Se antioxidant properties. It is also possible that Se can affect other aspects of vitamin E metabolism and transport to target tissues.

GSH-Px activity in the liver of day old chicks depends on Se supplied in the maternal diet. Low dietary Se was associated with decreased Se in egg yolk; and as a result Se-GSH-Px activity in the liver of newly hatched chicks significantly decreased (Surai, 2002). An efficient carry-over of Se and vitamin E from hens to progeny was indicated by a significant increase in muscle Se, liver GSH-Px activity and vitamin E content at hatching (Hassan *et al.*, 1990). There was no difference in Se-GSH-Px activity in the liver in response to further increased Se supplementation (from 0.2 to 0.4 mg/kg), which probably means that inclusion of 0.2 mg/kg Se in the maternal diet provides enough Se to the egg and embryonic tissues to meet the requirement for the maximum Se-GSH-Px activity. GSH-Px activity in the liver increased throughout embryonic development reaching maximum at time of hatching. In the liver of the newly hatched chick, Se-dependent GSH-Px is the major form of the enzyme comprising about 61% of total activity. In the majority of the tissues of the newly hatched chick there was a highly significant correlation between Se level and the activity of Se-GSH-Px.

In our study it has been shown that the effect of Se in the maternal diet is still apparent at 5 and 10 days of postnatal development. This finding suggests that Se accumulated in the liver of newly hatched chicks is actively used during the first days of postnatal development. It is possible to suggest that Se assimilation from the diet is low just after hatching and the chick relies on the reserves of the element accumulated during embryogenesis (Surai, 2002). Furthermore, our recent data from quail experiment indicate that increased Se levels in the diet as a result dietary organic Se supplementation are associated not only with increased Se level in tissues of newly hatched quail, but also at day 7 and 14 posthatch (Surai et al., not published).

Postnatal development of the chick is associated with changes in the antioxidant defence strategy. The main antioxidant protection of newly hatched chicks is afforded through high concentrations of natural antioxidants, mainly vitamin E and in some cases (wild birds) carotenoids in tissues. However, during the first 10 days post-hatch, vitamin E and carotenoid concentrations in the chicken liver decreased 20-fold; and the same is true for turkeys, ducks and geese. Therefore to compensate for this decrease in antioxidant potency, activity of GSH-Px in the liver significantly increased. As a result, this Se-dependent enzyme becomes the major player in antioxidant defence during postnatal development of the chicken.

Our data indicate that Se supplementation of the breeder diet at 0.2-0.4 ppm in the form of organic Se could provide substantial protection against free radicals and associated toxic metabolites to newly-hatched chicks. The benefit of organic Se use in breeder's diet lies in its efficient absorption, transport and accumulation in egg and embryonic tissues. This results in enhanced antioxidant status of the newly hatched chick. Since after hatching the levels of major natural antioxidants (vitamin E and carotenoids) in tissues progressively declines, the antioxidant enzymes are a critical part of antioxidant defence. Therefore enhanced GSH-Px activity in tissues as a result of organic Se supplementation of the maternal diet could be considered an effective means of increasing chick viability post-hatch. Enhancing antioxidant system capacity may also enhance immune response, which is extremely important at this period of chicken development.

In general there is some evidence to show that in commercial conditions inclusion of organic selenium into the breeder's diet is associated with improved hatchability. Furthermore organic selenium supplementation of the maternal diet decreased chick mortality for the first two weeks posthatch confirming a vitality of the idea of relationship between antioxidant defences and chicken viability (Surai, 2002).

Antioxidants and immune system

The immune system of the animal is based on natural and adaptive immunity (Figure 2). The natural immunity is dependent on the efficient function of phagocytic cells namely neutrophyls and macrophages which use free radicals as a weapon to kill pathogen, however, when escape from phagosome the same free radicals bacame dangereous and can damage all sort of biological molecule compromising phagocyte function and damaging adaptive immunity. Phagocytes also produce so called 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 are producing antibodies to specific non-self substances (B-lymphocytes) or directly attached to them (T-lymphocyte) and removed them from the cell. As one could realise that communication between those cells is a crucial factor of immunocoppetens. If we imagine that immune system is an army fighting against invaders (microorganisms, viruses, etc) than we would expect them to have something like mobile phones to receive and send signals to each other. Remarkably enough, major immune cells (macrophages, neutrophyls, T- and B-lymphocytes) have on their surface something like “mobile phones” called receptors. Those receptors are extremely sensitive to communicating molecules, but they also sensitive to free radicals and can be easily damaged. In such a situation without proper communication all those huge armies of immune cells would become useless. They also can start fighting each other as well as eventually destroying immunocompetence. If we imagine that immune cells are soldiers using chemical weapon to kill enemy, than special ammunition protecting them from their own weapon would be a crucial for effective battle. In the case of immune cells such ammunition is represented by natural antioxidants with Se-GSH-Px being a major defence. Based on the presented model it is clear that antioxidant defence is a crucial factor of immune defence in the body.

There is a great body of information confirming this idea. In fact Se- and vitamin E deficiencies are 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, Se supplementation is shown to improve immunocompetence and increase resistance to various diseases. This is true for variety of farm and companion animals including poultry, cows, sheeps,

horses, pigs, fish, cats and dogs. A summary of the effect of comptromised antioxidant system on immune system is shown in Figure 3.

Other antioxidant applications in poultry production

- Increased antioxidant supplementation for improvement meat quality during storage. It is proven for vitamin E (Sheehy et al., 1997) and vitamin E and selenium combination is even more effective (Surai and Dvorska, 2002l 2002a). Organic selenium is proven to decrease drip loss (Edens, 1997).
- Increased vitamin E supplementation to prevent heat stress-related decline in egg production (Bollengier-Lee et al., 1998; 1999).
- Decreased mycotoxin toxicity as a result of increased antioxidant supplementation (Surai, 2002).

A unique place of selenium in poultry production

From information presented above it is clear that Se is an important element in maintaining antioxidant system efficiency. It is widely accepted that Se works in the animal and human body as an integral part of a verity of selenoproteins. In fact Se in the form of selenocystein forms an active centre of the selenoproteins giving them unique properties, for example, antioxidant functions. First selenoprotein called glutathione peroxidase (GSH-Px) was identified in 1973. Since than the family of selenoproteins is becoming bigger every year and now it is generally accepted that there are more that 20 selenoproteins in animal and human body performing various important functions (Figure 4) including antioxidant defence, redox regulation of gene expression, thyroid hormone metabolism and structural role in spermatozoa.

Those selenoproteins can be active only when Se is supplied with the diet. There are two major forms of selenium for animal nutrition. Selenomethionine or organic selenium is the major form of selenium in various feedstuffs, including grains and oil seeds. Up to now a supplemental form of selenium used in animal industry is inorganic selenium, mainly selenite or selenate. Analyses of evolutionary aspects of animal nutrition (Surai and Dvorska, 2001) showed that during evolution animals had selenomethionine as a major form of selenium in their diet and as a result their digestive system was adapted to this form of selenium and there are principal differences in assimilation and metabolism of organic and inorganic selenium. For example, selenite is passively absorbed in the intestine as a mineral, used for immediate synthesis of some selenoproteins and the rest is released from the body with faeces and urine. In contrast, organic selenium is absorbed as an amino acid, similarly to methionine. A part of it is used for immediate synthesis of selenoproteins, similarly to selenite, but other part of the organic selenium is incorporated in newly synthesised proteins deposited, for example in muscles or eggs, and as a result Se reserves in the body are build. Therefore the main advantage of organic selenium is coming from better retention in the tissues and providing Se reserves for the body. These reserves are especially important in stress conditions when Se requirement increases but Se supply usually decreases as a result of decreases feed consumption. This is not the case when inorganic selenium is used in the diet since animals are not able to synthesis selenomethionine (Schrauser, 2000).

SeMet accumulated nonspecifically in muscle proteins can build selenium reserves which can be used in stress conditions when Se requirement increased but feed consumption usually decreased. In stress conditions protein catabolism by proteasomes can release SeMet which could serve as a source of Se for newly synthesied selenoproteins, such as GSH-Px, Thioredoxin reductase and Methionine sulphoxide

reductase. Those enzymes can deal with overproduction of free radicals and prevent decrease in productive and reproductive performance of farm animals. It was proven that selenium from both selenite and SeMet is readily available for synthesis of the selenoenzyme GSH peroxidase in rat tissues. There are also several lines of evidence confirming the idea that selenium accumulated in tissues in the form of SeMet can be available for selenoprotein synthesis.

First, our previous results (Surai, 2000) indicate that chicks hatched from eggs enriched with selenium by means of using Sel-Plex had higher GSH-Px activity in their liver not only at hatching, but more importantly, even at 5 days posthatch. This could be explain by usage of SeMet accumulated in tissues as a result of Se transfer from the egg during embryogenesis. Secondly, the bioavailability of the Se pool in maintaining liver GSH-Px activity during a period of Se deprivartion, following excess selenite or SeMet loading was assessed in rats (Ip and Hayes, 1989). In this study half-life of decay of the enzyme was calculated to be 4.2 and 9.1 days respectively, in those rats that had already been exposed to 3 ppm Se as either selenite or SeMet. Thirdly, in a human study Persson-Maschos et al. (1998) showed that in individuals who had been supplemented with organic selenium, the decline in the level of selenoprotein P after the end of a period supplementation was slower than in individuals who had been supplemented with selenite. Fourthly, when wheat and selanate were used as selenium sources in a supplementation study in Finnish men it was shown that once the supplements were withdrawn, platelet GSH-Px activity declined less in the group given wheat (Levander, 1983). Finally, several weeks' supplementation with high-Se bread increased New Zealand subjects' plasma selenium from 50-70 ng/ml to 120-175 ng/ml (Robinson et al., 1985). Plasma Se remained elevated when supplementation ceased. These data are in line with a suggestion that SeMet is the major selenocompound found initially in animals given this selenoamino acid, but is converted with time afterwards to selenocysteine (Whanger, 2002). For example, whole-body retention of ^{75}Se was greater for [^{75}Se]SeMet than for [^{75}Se]selenocystine but after the 1st week it decreased at a similar rate in both groups. The initial utilization of [^{75}Se]selenocystine was different from that of [^{75}Se]SeMet. However, after the 1st week ^{75}Se from both sources appeared to be metabolized similarly, suggesting that dietary Se of both forms is ultimately incorporated into the same metabolic pool (Thomson et al., 1975). In general SeMet has a slower, whole-body turnover in comparison to sodium selenite and there is greater efficiency in the reutilization of selenium from SeMet (Swanson et al., 1991).

We need to remember that selenomethionine is a storage form of selenium in the body. During protein catabolism in proteosomes selenomethionine can be released and used for additional selenoprotein synthesis and this could result in maintenance of high productive and reproductive performances of farm animals or good health of companion animals in stress conditions.

Let’s consider two different scenarios of antioxidant defence in poultry. First scenario, most common one, is for animals when inorganic selenium in the diet is used. In this case when stress conditions happened, the body tries to respond to them by using antioxidant reserves in the body and more importantly, by synthesising additional selenoproteins. In this scenario the main limitation would be absence of selenium reserves in the body and a restricted ability to synthesise additional selenoproteins and inadequate antioxidant protection when overproduction of free radicals occurs as a result of stress conditions. Therefore in this scenario we would expect immune system and general health to be compromised and reproductive success decreased. It is necessary to realise that we are not speaking about dramatic differences, this is sometimes a small difference difficult to notice, but after several consecutive stresses it could dramatically effect animal behaviour and health. This is especially important for newly born animals

including birds, since their antioxidant system is not mature and depends on antioxidant transfer from mothers through egg (birds) or colostrum and milk (mammals). Since inorganic selenium is not well transferred to the egg and even less effective in transferring to the milk we would not expect an antioxidant system improvement through this route in this scenario.

Another scenario could be the case when organic selenium is used. The major benefit would come from selenium reserves accumulated in the form of selenomethionine in muscles. When stress conditions happened during protein catabolism by proteosomes Se could be released and effectively used for the synthesis of additional selenoproteins to prevent damaging effect of free radical overproduction. This is especially important since many stresses are associated with decrease in feed consumption. As a result of such syntheses, selenoproteins could prevent to some extent lipid peroxidation and animals would benefit from it by maintenance of immunocompetence and reproductive performances. Since selenomethionine is transferred to the egg and to the milk and colostrum, newly hatched chicks or newly born animals will benefit very much as a result of improvement of their antioxidant system.

We need to realise that this scenario has limitations in terms of level of stresses we are considering. For example, when very high levels of toxins present in the diet or environmental stresses are too high the body response would not be sufficient to prevent pathobiological changes in animal body. On the other hand, there is a range of everyday stress conditions where this model/scenario could be effective.

Conclusions

Human, chickens, pigs, cows or any other animal species are exposed to free radical attack in everyday life and that is why an integrated antioxidant system has been developed in every cell during evolution to prevent damages to biologically relevant molecules including DNA, proteins and lipids. Some of antioxidants are synthesised in the body, however, major source of antioxidants is our diet. From many hundred dietary compounds possessing antioxidant activities selenium and vitamin E are considered to build a core of antioxidant defence. It has been appreciated that efficiency of antioxidants depends on their form in the diet. For example, in recent years it has been proven that organic selenium, for example in the form of Sel-Plex, has important advantages in comparison to inorganic selenium. It is interesting that the benefit of organic selenium has been proven practically for all species, including chicken, pigs, cows, sheeps and fish. It seems likely that an optimal combination of organic selenium and vitamin E in the diet is a key for an effective antioxidant defence. However, there is a need for further research in this field to establish those optimal combinations for each species depending on age, productivity and other relevant technological conditions.

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Table 1. Internal and external sources of free radicals

(Adapted from Surai, 2002)

Internally generated	External sources
Mitochondria	Cigarette smoke
Phagocytes	Radiation
Xanthine oxidase (EC 1.1.3.22)	UV light
Reactions with Fe and with	Pollution
other transition metals	Certain drugs
Arachidonate pathways	Chemical reagents
Peroxisomes	Industrial solvents
Exercise	
Inflammation	
Ischemia and reperfusion	

Figure 1. Three major levels of antioxidant defence in the cell

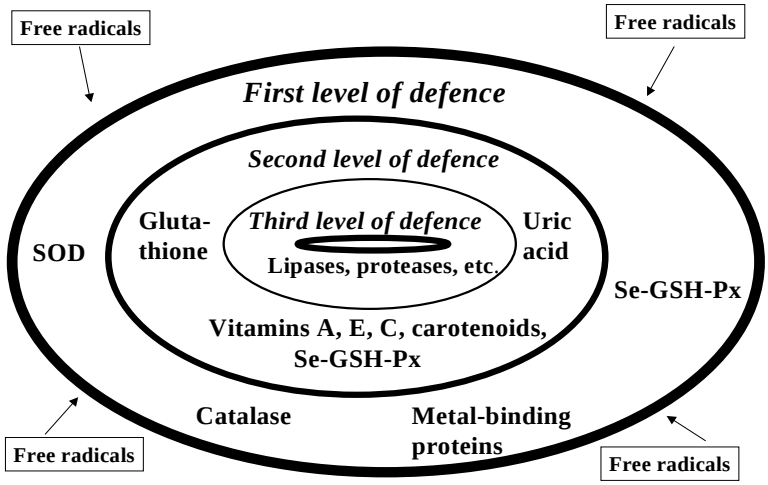

Figure 2. General scheme of the immune system

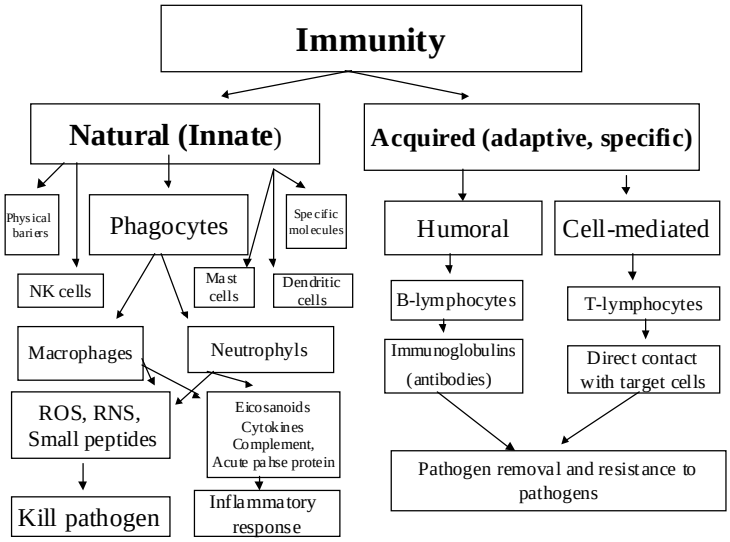

Figure 3. Oxidative stress and immune system

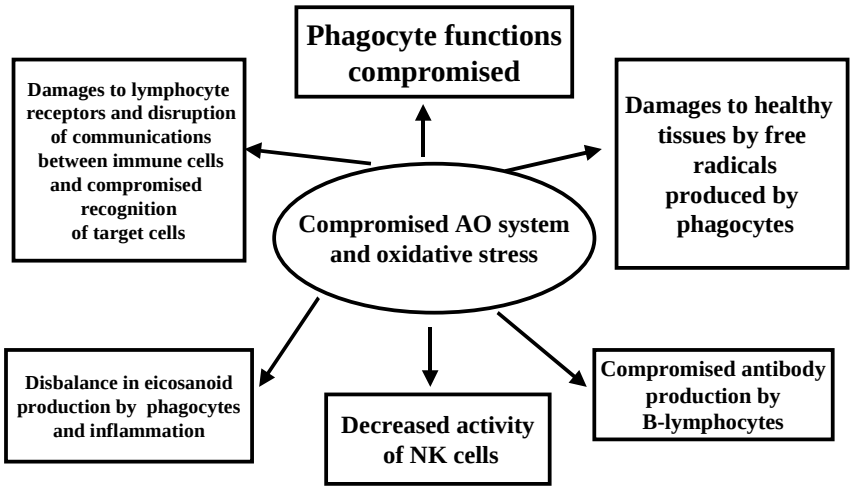

Figure 4. Selenoproteins in relation to poultry