Feeding breeders to avoid oxidative stress in embryos

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Introduction

Breeding programmes are designed to produce a broiler with high potential for growth, yield, and feed efficiency. These traits that may compromise health status under husbandry designed to maximise this genetic potential, since there is a trade-off between growth- and health-related traits with responses to husbandry programs, age, sex, and genetic line (Siegel *et al.*, 2001). In this respect it is well known that chick viability is an important factor determining profitability of the poultry industry. From fertilised ovum to the placement at the broiler farm, various factors, such as incubating egg quality, egg storage conditions, incubation conditions and conditions that exist between hatching and placement at the farm and their interactions are important determinants of chick quality (Decuypere *et al.*, 2001). In contrast to mammals, chicken embryonic development takes place in a semi-closed system, the egg, where only exchange of gas and water take place. It has been appreciated recently that the success of embryonic development depends on egg composition and conditions of egg incubation. Therefore a great body of evidence indicates that avian maternal nutrition is the major determinant of the health and development of the progeny similar to humans and other mammals. The egg composition is designed in such a way that all nutrients necessary for the development of the future embryo are accumulated within the egg yolk and white (Speake *et al.*, 1998). Among different nutrients in the maternal diet which could significantly affect chick embryo development and their viability in the early posthatch life, polyunsaturated fatty acids (PUFAs) and natural antioxidants have been suggested to play a central role. In fact, the high levels of endogenous antioxidants within the egg and embryonic tissues can clearly serve as a major adaptive mechanism for the protection of the tissue during the oxidative stress experienced at hatching.

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. 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 2×10^{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

(Surai, 2002, 2006, Fisinin and Surai, 2011)

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 living organisms have developed specific antioxidant protective mechanisms to deal with ROS and reactive nitrogen species (RNS). Therefore it is only the presence of natural antioxidants in living organisms which enable them to survive in an oxygen-rich environment. These mechanisms are described by the general term "antioxidant system". It is diverse and responsible for the protection of cells from the actions of free radicals. This system includes:

- natural fat-soluble antioxidants (vitamins A, E, carotenoids, ubiquinones, etc.);
- water-soluble antioxidants (ascorbic acid, uric acid, taurine, etc.)
- antioxidant enzymes: glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD).
- thiol redox system consisting of the glutathione system (glutathione/glutathione reductase/glutaredoxin/glutathione peroxidase and a thioredoxin system (thioredoxin/thioredoxin peroxidase/thioredoxin reductase).

The protective antioxidant compounds are located in organelles, subcellular compartments or the extracellular space enabling maximum cellular protection to occur. Thus antioxidant system of the living cell includes three major levels of defence:

The first level of defence is responsible for prevention of free radical formation by removing precursors of free radicals or by inactivating catalysts and consists of three antioxidant enzymes namely SOD, GSH-Px and CAT plus metal-binding proteins. Since the superoxide radical is the main free radical produced in physiological conditions in the cell, superoxide dismutase (EC 1.15.1.1) is considered to be the main element of the first level of antioxidant defense in the cell. This enzyme dismutates the superoxide radical in the following reaction:

$$2O_2^{\bullet} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

The hydrogen peroxide formed by SOD action can be detoxified by GSH-Px or CAT which reduce it to water. Transition metal ions also accelerate the decomposition of lipid hydroperoxides into cytotoxic products such as aldehydes, alkoxyl radicals and peroxyl radicals. Therefore, metal-binding proteins (transferrin, lactoferrin, haptoglobin, hemopexin, metallothionenin, ceruloplasmin, ferritin, albumin, myoglobin, etc.) also belong to the first level of defence. It is necessary to take into account that iron and copper are powerful promoters of free radical reactions and therefore their availability in "catalytic" forms is carefully regulated *in vivo*. Therefore organisms have evolved to keep transition metal ions safely sequestered in storage or transport proteins. In this way the metal-binding proteins prevent formation of hydroxyl radical by preventing them from participation in radical reactions.

Unfortunately this first level of antioxidant defence in the cell is not sufficient to completely prevent free radical formation and some radicals do escape through the preventive first level of antioxidant safety screen initiating lipid peroxidation and causing damage to DNA and proteins. Therefore the second level of defence consists of chain-breaking antioxidants - vitamin E, coenzyme Q, carotenoids, vitamin A, ascorbic acid, uric acid and some other antioxidants. Glutathione and thioredoxin systems also have a substantial role in the second level of

antioxidant defence. Chain-breaking antioxidants inhibit peroxidation by keeping the chain length of the propagation reaction as small as possible. Therefore, they prevent the propagation step of lipid peroxidation by scavenging peroxyl radical intermediates in the chain reaction.

However, even the second level of antioxidant defence in the cell is not able to prevent damaging effects of ROS and RNS on lipids, proteins and DNA. In this case, the third level of defence is based on systems that eliminate damaged molecules or repair them. This level of antioxidant defence includes lipolytic (lipases), proteolytic (peptidases or proteases) and other enzymes (DNA repair enzymes, ligases, nucleases, polymerases, proteinases, phospholipases and various transferases).

All these antioxidants are operating in the body in association with each other forming an integrated antioxidant system. The co-operative interactions between antioxidants in the cell are vital for maximum protection from the deleterious effects of free radicals and toxic products of their metabolism. For-example, it is well established that vitamin E is the major antioxidant in biological membranes, a "head quarter" of antioxidant network. However it is usually present there in low molar ratios (one molecule per 2000-3000 phospholipids) but vitamin E deficiency is difficult to induce in adult animals. It is probably due to the fact that oxidised vitamin E can be converted back into the active reduced form by reacting with other antioxidants: ascorbic acid, glutathione, ubiquinols or carotenoids. Therefore the antioxidant protection in the cell depends not only on vitamin E concentration and location, but also relies on the effective recycling. Indeed, if the recycling is effective then even low vitamin E concentrations are able to maintain high antioxidant protection in physiological conditions. Antioxidant recycling is the most important element in understanding mechanisms involved in antioxidant protection against oxidative stress. The rate of regeneration, or recycling, of the vitamin E radicals may affect both its antioxidant efficiency and its lifetime in biological systems. As can be seen from data presented above the antioxidant defence includes several options:

- Decrease localised oxygen concentration;
- Prevention of first-chain initiation by scavenging initial radicals (SOD, GSH-Px and catalase);
- Binding metal ions (metal-binding proteins);
- Decomposition of peroxides by converting them to non-radical, non-toxic products (Se-GSH-Px);
- Chain-breaking by scavenging intermediate radicals such as peroxyl and alkoxyl radicals (vitamins E, C, glutathione, uric acid, ubiquinol, bilirubin etc.);
- Repair and removal of damaged molecules. Additional defensive mechanisms responsible for maintenance of physiological metabolism in stress conditions include:
- Antioxidant recycling mechanisms;
- Redox-signalling and vita gene expression with an additional synthesis of important antioxidant molecules including stress-protein synthesis (e.g. heat shock proteins, etc.);
- Apoptosis (can remove damaged cells and restrict mutagenesis)

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 **(Figure 1)**. 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, carotenoids and coenzyme Q provide antioxidant defence in lipids, specifically biological membranes. The function of each is important to the effectiveness of the other antioxidants. For example, vitamin E potency is very much dependent on the efficacy of its recycling. Indeed, vitamin C 'recycles' vitamin E from oxidized

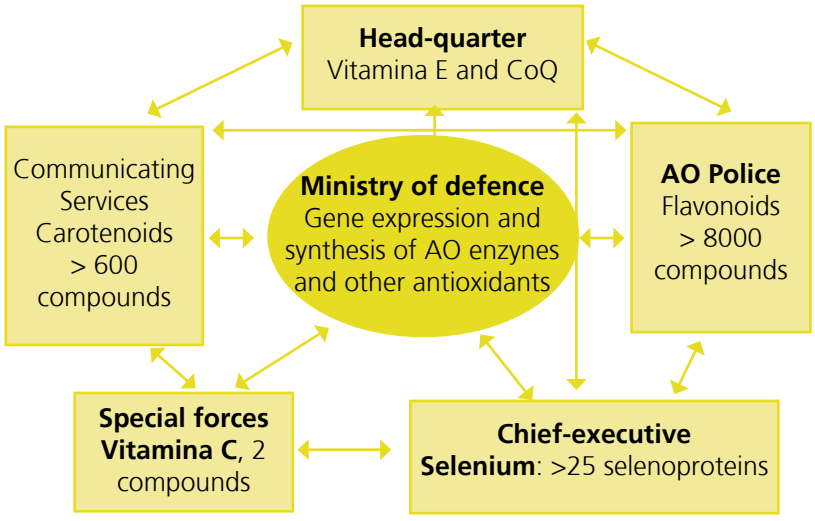

Figure 1 - Antioxidant system.

form to become active again, while glutathione does the same for vitamin C. Furthermore, vitamins B1 and B2 are also involved in vitamin E recycling. 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 dramatically increases, then without an external help it is difficult to prevent damages to major organs and systems. This external help takes the form of increased dietary supplementation of natural antioxidants, especially vitamin E, carotenoids and selenium. For the nutritionist or feed formulator the challenge is to understand when and how much external 'help' is needed and economically justified.

Antioxidant defences of the developing embryo

(Surai, 2002; 2005, 2006)

For a precocial species like the chicken, metabolic rate and oxygen consumption increase rapidly during and just after hatching to meet the demands of endothermy and locomotion, supported by the transition from chorioallantoic to pulmonary respiration (Hohtola and Visser, 1998). To cope with this sudden exposure to oxidative stress, it would clearly help if an effective antioxidant system was already in place. Furthermore, chick embryo tissues contain a high proportion of polyunsaturated fatty acids in the lipid fraction which are highly susceptible to lipid peroxidation. That is why tissues of newly hatched chicks express a range of antioxidant defence mechanisms, including natural antioxidants (vitamin E, carotenoids, ascorbic acid, reduced glutathione, coenzyme Q, etc.) and enzymes (superoxide dismutase, glutathione peroxidase and catalase) as well as mineral cofactors (Se, Zn, Mn and Fe). Vitamin E, carotenoids, Se, Cu, Fe and manganese are delivered from the maternal diet via the egg and the others are synthesised in the tissues. 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 minerals such as selenium are transferred from feed into egg yolk and further to embryonic tissues. Research indicates that increased supplementation of the maternal diet can substantially increase vitamin E, carotenoid and Se concentrations in developing chick tissues and significantly decreases susceptibility to lipid peroxidation.

Postnatal development of the chick is associated with changes in the antioxidant defence strategy. The main protection from oxidation in 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. To compensate for this decrease, activity of GSH-Px in the liver significantly increases. As a result, this Se-dependent enzyme becomes the major player in antioxidant defence during postnatal development of the chicken.

Vitamin E in egg yolk and embryo

(Surai, 1999, 2002)

Vitamin E is considered to be the main antioxidant in egg yolk, which is delivered from the feed and transferred to the developing embryo. The main points of vitamin E metabolism in avian species include:

- Main chain-breaking antioxidant
- Located in biological membranes and lipid droplets
- Vitamin E exists in nature in 8 different forms including 4 tocopherols and 4 tocotrienols
- Alpha-tocopherol possesses the highest biological activity
- Absorbed in small intestine with efficiency depending on diet composition, level of supplementation, age, sex and other individual characteristics
- Accumulated to some extent in the liver and adipose tissues, however, accumulation is not sufficient to meet long-term requirements.
- Crude plant oils are the richest source of vitamin E, however, oil refining decreases vitamin E concentration dramatically.
- In poultry industry vitamin E concentration in feed ingredients is not counted and supplemental vitamin E is the main source of the vitamin for poultry
- The recommended vitamin E supplementation of breeders (Cobb and Ross) is 100 ppm
- Increased (up to 250 ppm) vitamin E supplementation of laying hen in stress conditions is shown to decrease detrimental consequences of heat stress
- Unstable and easily oxidised. Commercial preparations usually contain esterified forms

of the vitamin (eg. 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
- Non-toxic. Even very high doses are not associated with hypervitaminosis
- Comparatively expensive
- Vitamin E is effectively transferred to the egg yolk and further to the developing embryo
- With a single egg a laying hen releasing more vitamin E than all its reserves in the liver.
- Eggs from wild birds are characterised by comparatively high levels of vitamin E concentration
- There are species-specific differences in vitamin E accumulation in avian eggs with chicken egg to be richer in this vitamin in comparison to turkey, goose or duck eggs obtained from breeders supplemented with the same amount of vitamin E
- The highest vitamin E concentration is found at time of hatching and vitamin E accumulation in the embryonic liver is considered to be an adaptive mechanism providing antioxidant defence at critical time of hatching
- For the first 2 weeks post-hatch there is a dramatic (10-20-fold) reduction in vitamin E concentration in the chicken liver
- Vitamin E concentration in the egg determines vitamin E status of the chick at least for the first week post-hatch
- Newly hatched chicks are not able to assimilate vitamin E from the diet effectively and are dependent on its reserved built during embryonic development
- Increased vitamin E dietary supplementation of the newly hatched chick has limited ability to increase their vitamin E status
- Increased dietary vitamin E supplementation of the maternal diet was associated with increased vitamin E concentrations in the egg yolk, embryonic tissues and their increased resistance to oxidative stress

It seems likely that an increased vitamin E supplementation of breeders (>100 ppm) in commercial conditions is not always associated with improved their performance and egg quality. For example, in recent studies there were no differences in hatchability of eggs obtained from laying supplemented with 15, 150 or 300 ppm vitamin E (Buswas *et al*., 2010) or 40, 80 or 120 ppm vitamin E (Tsai *et al*., 2008).

Carotenoids in egg yolk and embryo

(Surai, 2002; Surai *et al*., 2003: Surai, 2012)

Recently natural carotenoids have been included into the category of antioxidants. They could be characterised as follows:
- Pigments responsible for yellow, orange and sometimes red pigmentation in plants, insects, birds and marine animals. Chicken egg yolk pigmentation is due to presence of carotenoids
- Working in the body as an integral part of the antioxidant system, providing recycling other antioxidants including vitamin E
- Easily accumulating in the egg yolk and transferring to the developing embryo
- During embryonic development part of accumulated in egg yolk carotenoids are used for metabolic needs including antioxidant defences
- Many avian species in wild are characterised by comparatively high (5-10-times higher than commercial eggs) carotenoid concentrations
- Eggs obtained from breeders fed on maize-based diet contain at least double carotenoid concentration in comparison to eggs from laying hens fed on wheat-barley diet
- Have some health-promoting properties, including immune system modulation. It was proven that carotenoid-dependent bright coloration of plumage in males in various avian species in wild is indicative of their health, in particular effective immunity
- There are no established requirements for animals.
- Many plant-derived foods are rich in carotenoids, but animal-derived foods are poor sources
- Unstable and oxidised during feed storage
- Reserves in the body are limited
- During embryonic development carotenoids are actively transferred from egg yolk to the developing embryo with maximal concentration in the chick liver at hatching time. Similarly to vitamin E this accumulation is considered to be an adaptive mechanism to deal with hatch-related stress conditions.
- Similarly to vitamin E, after hatching carotenoid concentration in the chick liver dramatically decreased
- Chicks obtained from carotenoid-depleted eggs are not able to assimilate carotenoids from the diet to the same extent as those

hatched from carotenoid-rich eggs

- Increased dietary carotenoid supplementation of the maternal diet was associated with increased vitamin E concentrations in the egg yolk, embryonic tissues and their increased resistance to oxidative stress.

Among more than 750 known carotenoids, canthaxanthin has a special place as a carotenoid with proven antioxidant and other biologically-relevant functions. A great body of evidence indicates that canthaxanthin possesses high antioxidant activity that was shown in various *in vitro* model systems as well as in animal experiments *in vivo*. Recently effects of canthaxanthin in the breeder's diet have been studied (Surai *et al*., 2003). The control group received the control diet containing < 2 mg/kg total xanthophylls. For treatments 2 to 5 the control diet was supple¬mented with 3, 6, 12 and 24 mg/kg canthaxanthin respectively. The control diet did not contain canthaxanthin.

Inclusion of canthaxanthin into the maternal diet caused a significant dose-dependent response in terms of its accumulation in the egg yolk. Egg yolk colour gradually changed from pale yellow in the control group to dark red in the laying hens fed canthaxanthin at 24 mg/kg. The most important finding of this study was a positive effect of canthaxanthin on vitamin E in the developing chicks. As a result of enrichment of the chick liver with canthaxanthin and vitamin E due to the maternal dietary supplementation tissue susceptibility to lipid peroxidation significantly decreased. This was the case in 1-d-old and 7-d-old chicks. In a later study it was shown that maternal diet is the main determinant of the progeny's carotenoid status for at least 7 days after hatching (Karadas *et al*., 2005). The enhancement of the antioxidant system of the developing chick as a result of feed additives to maternal diet presents great opportunities for poultry producers. Postnatal development of the chicken is a crucial time for the maturation of major physiological systems, including the immune system, as well as a time of high risk of peroxidation (Surai, 2002). Therefore, increased supplementation of the maternal diet with carotenoids, in particular canthaxanthin, could help maintain antioxidant system efficiency and high chick viability.

The idea of beneficial effect of dietary CX supplementation of the breeder diet was further developed by Chinese authors (Zhang *et al*., 2011). In this study the breeder hens were fed either a basal diet or the basal diet supplemented with 6 mg of canthaxanthin/kg for 24 wk. Canthaxanthin supplementation resulted in a significant increase in the yolk colorimetric score of Roche Yolk Color Fan ($P < 0.001$), confirming an effective CX transfer from the diet to the egg yolk. Egg yolk enrichment with CX was associated with a significant improvement of the antioxidant status of the egg yolk ($P < 0.05$). Indeed, MDA content of the egg yolk decreased from 139.83 nmol/g down to 86.92 nmol/g (P=0.023). At the same time total antioxidant capacity (TAC) of the egg yolk increased from 1.87 U/g up to 3.16 U/g (P< 0.001). This could be an important element in an improvement of protection of laying birds from commercially-relevant stresses (Surai, 2002). The antioxidant capacity of the newly hatched chicks was significantly increased ($P < 0.05$) due to maternal CX supplementation. There was a significant decrease in MDA (from 4.28 down to 2.61 nmol/ml, P<0.001) in plasma of day-old chicks. It is of great importance to see an increased (from 98.4 to 144.7 U/ml, P<0.05) SOD activity in the chicken plasma. It is interesting to underline that maternal supplementation with CX has also positive effect on TAC of newly hatched chicken which increased by 33% (from 13.8 to 18.3 U/ml, P= 0.052).

In a field trial, Robert *et al*., (2008) studied the effect of canthaxanthin in ROSS breeders on the anti-oxidant status of their progeny. They observed that the antioxidant status of sera of 1-day old chicks was significantly higher and the TBARS level significantly lower with 6 ppm canthaxanthin in the breeder feed. The experimental chicks also showed a lower mortality (0 *vs*. 4%) during the first 21 d posthatching. These findings support the hypothesis that CX supplementation of the maternal diet enhances the protective capacity of tissues against oxidative stress in vivo, which might be beneficial for poultry producers.

The next step in proving a beneficial effect of CX supplementation of the maternal diet was an experiment conducted in Brazil in the Poultry Science Laboratory of the Department of Zootechnics at the Federal University of Santa Maria (Rosa *et al*., 2012). In terms of breeder performance, canthaxanthin was observed to significantly improve egg laying rate in certain periods, but overall there was no difference between the two treatment groups. Similarly to previous observations the reduction of TBARS was observed in yolks from stored hatching eggs produced by breeders fed diets plus canthaxanthin. This reduction was observed in eggs submitted to analysis on the same day they were produced (P<0.05) and in eggs stored for four (P<0.001), eight (P<0.001) and twelve days (P<0.0001).

The most important finding of this study was a positive effect of CX supplementation of the maternal diet on fertility, hatchability and embryonic mortality. In fact, canthaxanthin in comparison to the control improved fertility (92.1 *vs*. 91.0%, P<0.02) and hatchability (93.7 *vs*. 91.3%, P=0.0003) and reduced embryonic mortality (3.7 *vs*. 5.5%, P<0.003). As a result, hatching rate was significantly (86.2 vs. 83.0%, P=0.0001) improved. As to the different stages of embryo mortality, canthaxanthin was most efficacious for its prevention in the first 48 hours (1.04 *vs*. 1.8%, P= 0.008) as well as between day 15 and 21 of incubation (1.44 *vs*. 2.07, P=0.017). In the study, canthaxanthin supplementation improved breeder fertility (P=0.0171) perhaps by improving survival and storage of spermatozoa within the reproductive tract of the hen. It is interesting to mention that another carotenoid with a similar structure astaxanthin (AX) fed to breeders was shown to be transferred to the egg yolk and had a significant protective effect against decreasing hatchability due to egg storage at 21 °C (Saito and Kita, 2011). In the study the hatchability of eggs stored at 21 °C was significantly lower than that at 10 °C, and lowered hatchability was restored by maternal intake of AX. Elevated levels of dietary AX from 5 to 20 ppm gradually restrained the decrease in hatchability at 21 °C.

Selenium in egg yolk and embryo

(Surai, 2002, 2005, 2006; Fisinin *et al*., 2008)

The main characteristics of selenium include:

- It is an essential part of a range of selenoproteins, including glutathione peroxidase (GSH-Px), thioredoxin reductase (TrxR), iodothironine deiodinase (ID) and some others. In the animal and human body 25 selenoproteins have been identified to date
- 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. However, the doses of dietary Se which could be detrimental are 10-20-fold higher in comparison to those commercially used
- There are two major sources of Se for poultry: a natural source in the form of various selenoamino acids including selenomethionine (Se-Met) or inorganic selenium in the form of selenite or selenate
- It is proven that organic selenium supplementation has physiological and biochemical benefits in animals, including poultry
- Se concentration in the egg yolk and egg white depends on its concentration and form in the diet. The main form of selenium in the egg is Se-Met, which cannot be synthesised by laying hens. Therefore inclusion of organic selenium (selenized yeast or Se-Met) in the chicken diet is associated with a substantial increase Se concentration in the embryonic tissues (Surai, 2000; Yuan *et al*., 2011).
- Increased Se concentrations in the egg yolk and albumin are related to increase Se levels in the embryonic liver and increased antioxidant defences of the developing chicks
- The order of Se distribution was liver > kidney > spleen > cardiac muscle > egg > blood > breast muscle, irrespective of the addition level or source (Pan *et al*., 2007)
- Maternal Se has a long-term consequences for the developing embryo and newly hatched chicks (Pappas *et al*., 2005).
- It seems likely that Se accumulated in the egg can affect gene expression of the developing embryo and this could result in better antioxidant defences and reduced Se use during development or alternatively specific enzymatic systems involved in the Se metabolism could be affected and Se would be more effectively assimilated from the diet.

Other antioxidants

Since ascorbic acid is synthesized in the chicken body and not found in the egg its direct role in egg quality is questionable. The results of Creel *et al*., (2001) did not provide evidence of a beneficial reproductive response to the inclusion of ascorbic acid in commercial broiler breeder diets. However, in stress conditions ascorbic acid supplementation of the breeder's diet could have some positive effects. It is interesting to mention that, L-carnitine, possessing antioxidant properties, in the diet of hens affected carcase traits of their progeny. In particular, dietary hen L-carnitine decreased carcase fat and increased breast meat in progeny fed on high nutrient density diets (Kidd *et al*., 2005). Furthermore, dietary L-carnitine, as compared with the control diet, increased egg yolk L-carnitine concentration (P = 0.001), decreased hatchling yolk sac weights (P =

0.0001), decreased yolk sac lipid content at hatch (P = 0.01), and culminated in compositional changes of yolk fatty acids (Zhai *et al*., 2008). There were several attempts to improve hatching egg quality by inclusion into the breeder's diet various plant extracts, essential oils and some other compounds possessing antioxidant properties. However, it is too early to make any conclusion about their efficacy.

Maternal effects of antioxidants

It is generally accepted that the quality of newly hatched chicks depends on the egg composition. However, recent developments in the areas of maternal programming and gene expression, indicate that maternal affects can be seen further into the postnatal development of chicks, than previously thought. For example, increased Se concentration in the quail egg was associated with increased Se concentration in the liver, brain, breast and leg muscles of newly hatched quail (Surai *et al*., 2006). This difference was shown to be significant for 2 weeks post-hatch. It has therefore been suggested, that the maternal effect of dietary selenium can be seen beyond the time of hatching and more importance should be given to this effect. Similar results have been seen with broiler breeders. In a study conducted at the Scottish Agricultural College measured the extent to which the effect of dietary supplementation of breeder diets with Se, continue onto the next generation (Pappas *et al*., 2005). Hens were maintained on control or Se-supplemented diets, containing 0.027 and 0.419 µg Se/g of feed, respectively. The high-Se diet elevated the Se content of the hens' eggs by 7.1 times. At hatch, the concentrations of Se in the liver, breast muscle and whole blood of the chicks originating from the high-Se parents were, respectively, 5.4, 4.3 and 7.7 times higher than the values in the chicks of the low-Se parents. When the offspring from the two parental groups were maintained on the low-Se progeny diet, the tissue Se concentrations in chicks originating from the high-Se hens remained significantly higher for 3-4 weeks after hatching, compared to the values found in chicks from the low-Se hens. Similarly, tissue glutathione peroxidase activity remained significantly higher in chicks from the high-Se hens for 2-4 weeks post-hatch. Thus, it was concluded that the effects of maternal Se supplementation persist in the progeny for several weeks after hatching. Probably vitamin E in breeder diets can also affect progeny. Indeed, it was shown that Vitamin E supplementation of breeder birds increased the immune response of their progeny (Haq *et al*., 1996). Similarly, antibody titres against Newcastle disease virus (NDV) or avian influenza virus (AIV) and the plasma concentration of interleukin (IL)-1 were increased in progeny chicks obtained from laying hens supplemented with 100 ppm vitamin E in comparison to 0 or 40 ppm supplementation (Zhao *et al*., 2011).

Recently, the effect of fortifying breeder diet with vitamins and minerals on gene expression in the intestine of progeny has been investigated (Rebel *et al*., 2006). Unfortunately the authors did not report the egg composition before and after dietary fortification but from the data reported one could expect substantially increased levels of vitamin E in the egg and slightly increased levels of vitamins A, D, B1 and B2, as well as selenium. Gene expression patterns in the intestine were measured at 3 and 14 days of age with an intestinal cDNA-microarray. Between the two groups, 11 genes were found to be differently expressed at both 3 and 14 days of age. Genes that are expressed differently affect intestinal turnover, cell proliferation and development, metabolism and feed absorption. Taking into account these data and results presented by Koutsos *et al*., (2003) showing maternal effect of carotenoids on their concentration in 4-week-old chickens; it is possible to suggest that various antioxidants including carotenoids could affect gene expression during the embryonic development. This could result in better antioxidant defences related to higher hatchability and better chicken viability posthatch. The authors showed that concentration of carotenoids in the egg yolk had a profound effect on the immune system development posthatch. They suggested the response to carotenoid-depletion in the egg yolk may compromise the capacity for an appropriate immune response later in the bird's life (Kuotsos *et al*., 2007). Indeed, this hypothesis needs further clarification; however, it is clear that maternal effect is seen beyond newly hatched chicks. In general this could be an example of maternal nutritional programming responsible for various changes in postnatal life of chicken. Indeed, the chicken egg could be an ideal model to study this phenomenon and more research should be carried out in this area. The prevailing nutritional environment during fetal development exerts powerful and long-lasting effects upon physiology and metabolism (Langley-Evance, 2009). Indeed, in mammals there are already several genes described which are specifically regulated by carotenoids, including CX (Zhang *et al*., 1992; for review Bertram, 1999; Stahl *et al*., 2002). It is just a matter of time before such genes are identified in chickens.

When broiler breeder diets were supplemented

with Se-Yeast, gene expression analysis revealed that the quantity of gene transcripts associated with energy production and protein translation were greater in the oviduct of the experimental birds in comparison to control breeders. Targets up-regulated by Se-Yeast, included genes encoding several subunits of the mitochondrial respiratory complexes, ubiquinone production and ribosomal subunits (Brennan *et al*., 2011). Dietary supplementation of the breeder diet with Se-Yeast for 4 weeks was associated with a significant increase in Se content of the muscle of the 21-day-old progeny chicks. Those changes were associated with decreased lipid and protein oxidation and muscle drip loss in comparison to chickens obtained from the breeders fed on a control diet without Se supplementation and containing feed-derived Se at 0.13 mg/kg (Wang *et al*., 2009). The same authors showed that feed utilization was better ($p<0.05$), and mortality was lower ($p<0.05$) in the progeny from hens fed with selenomethionine throughout the 8-week growing period compared with those from hens fed with sodium selenite (Wang *et al*., 2011).

In an experiment conducted in China, the influence of mineral sources on broiler breeders and their offspring was investigated. Broiler breeder hens were fed with diets containing either organic or inorganic trace minerals (Cu, Mn, Zn, and Se) at equal levels. Supplementations of organic minerals in breeders' diets were observed to have protective effects on breeders via increasing cholesterol and triglyceride clearance from plasma and decreasing plasma lipid peroxidation. Furthermore, increased body weight (2.247 vs 2.099, P<005) and feed efficiency (1.671 vs 1.704, P<0.01) were observed in chicks from breeders fed organic mineral diets for 42 days (Sun *et al*., 2011).

Antioxidants and stresses

(Surai, 2002; 2005; 2006; Fisinin and Surai, 2011)

When the antioxidant system finds itself in high stress conditions, if free radical production is increased dramatically, then without external help there will be difficult to prevent damage to organs and cells. Such external help can be provided by dietary supplementation with increased doses of natural antioxidants, including vitamin E, carotenoids and selenium. For nutritionists or feed formulators it is a great challenge to understand when the antioxidant team in the animal body requires help and how much of this help can justify extra feed expense, because antioxidants are typically expensive components of the diet.

A simplified list of possible relevant stresses in breeders includes the following:

- **Time** between an egg being laid and its cooling down for storage. Eggs should be collected frequently in hotter climates. In such conditions free radical damages to lipids and proteins can occur and antioxidant protection is beneficial.
- **Egg storage before incubation** often associated with lipid peroxidation within egg membranes, particularly those containing high levels of PUFAs. Increased dietary antioxidant provision can be an effective means to prevent damaging effects of free radicals produced within the egg.
- **Temperature, humidity and carbon dioxide concentration** fluctuations during incubation can affect embryonic development, oxidation and phosphorylation in tissues leading to free radical production. For example, high carbon dioxide concentrations during the incubation period has been shown to jeopardise the liveability of the embryo
- **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 polyunsaturated fatty acids (PUFA). At the same time natural antioxidant reserves have not reached a sufficient level for innate protection. At this stage of development 'piping' occurs; and oxygen availability for tissues increases. Low antioxidant status in combination with high temperature, humidity, and PUFAs can increase susceptibility to lipid peroxidation.
- **Hatching time** is considered as an environmental stress for the chick. At this point natural antioxidant concentrations have reached a maximum, but high levels of lipid unsaturation in tissues, decreasing concentration 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 heterogeneous nature 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 may be in the incubator for 2-12 hours longer than

others. This puts pressure on antioxidant defence capacity. Furthermore, any delay in food and/or water intake after hatching usually negatively affect a number of performance parameters and a delay occurs in the maturation of the enzymatic systems that control metabolism, free radical production and antioxidant protection systems. Recently we showed (Karadas *et al*., 2011) important tissue-specific changes in the concentrations of the major antioxidants (vitamin E and coenzyme Q10) during the 36 h post-hatch.

- **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 should be associated with temperature fluctuation and dehydration.

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.

It is proven that ROS formation, which is positively implicated in cellular stress response mechanisms, is a highly regulated process controlled by a complex network of intracellular signalling pathways. By sensing the intracellular redox status and energy status, the functional state of mitochondria, and the concentration of ROS produced in mitochondria, a special network regulates protective mechanisms by co-ordinating information flow along its convergent, divergent and multiply branched signalling pathways, including vitagenes which are genes involved in preserving cellular homeostasis during stressful conditions. Vitagenes encode for heat shock proteins, the thioredoxin, the sirtuin protein systems and some other antioxidant molecules. Many various dietary compounds, for example carnitine, are believed to be protective in stress conditions through the activation of various pathways, including vitagenes. Their roles in animals production and in chick embryonic development are not studied yet but a protection against stress is their vital function. Given the broad cytoprotective properties of products of vitagenes, there is now a strong interest in discovering and developing pharmacological agents capable of inducing the vita genes, providing maximum natural protection in stress conditions.

Our recent work related to understanding mechanisms of stress in farm animals and poultry and attempts to find a proper nutritional supplements to decrease negative consequences of the excess formation of free radicals in stress conditions has been finalised with a development a composition "Feed Food Magic Antistress Mix", containing 28 essential compounds and provided to poultry and farm animals with water in stress conditions (Surai and Fotina, 2010; Surai and Fotina, 2010a; Surai and Borodai, 2010). Protective effect of aforementioned antioxidant mixture has been successfully tested with broiler breeders in hot summer weather, with rearing birds before and after vaccinations (Fisinin and Surai, 2011a), during their transfer to breeding houses as well as with broilers in first days after placement (Fisinin and Surai, 2012), before and after vaccinations, in hot summer conditions, during mycotoxicoses, etc. (Fisinin and Surai, 2011b). Indeed, many years of the research devoted to antioxidants and stresses brought about new understanding and helped developing the effective means to decrease negative consequences of stresses on breeders, layers. rearing birds and broilers.

Conclusions

Evidence is accumulating indicating that a maternal diet can have a profound effect on the hatching egg quality and health status, growth and development of newly hatched chick. Several points are important to outline:

- Maternal diet determines chicken development during egg incubation and for the first few days post-hatch;
- Vitamin E and carotenoids accumulation in the chicken liver during embryonic development is considered as an adaptive mechanism to deal with stress conditions of the hatching process.
- In postnatal development a strategy of antioxidant defence is changed from antioxidant accumulation to the synthesis of antioxidant enzymes with more sophisticated and effective regulation at the gene level;
- Immune and digestive systems of the chicken are not mature at hatch and are actively developing during first 2 weeks post-hatch and are at risk of oxidative stress and need effective antioxidant protection;
- Increased vitamin E/carotenoid/Se supplementation of the maternal diet is proven to be beneficial for the embryonic and early postnatal development of the chick;
- A new antistress composition provided with drinking water is developed and successfully tested in commercial conditions of Ukraine and Russia.

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