Natural Antioxidants in Poultry Nutrition: New developments

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Introduction

It is increasingly appreciated that diet plays a pivotal role in maintaining animal health, productive and reproductive performance of farm animals and poultry. Among many dietary factors natural antioxidants have special importance in the maintenance of high growth levels, reproduction and immuno-competence in poultry production. This concept is based on understanding the contribution of antioxidants in reducing the detrimental effects of free radicals and toxic metabolites on animals.

Natural antioxidants in feed ingredients

It has been suggested that antioxidant-prooxidant balance in each cell and in whole body in general is responsible for a regulation of major physiological functions in the body. The anti-oxidant/pro-oxidant balance can be modulated by sub-optimal diets and poor nutrient intakes, or positively affected by dietary supplementation. Therefore, feed components can change this balance and may influence such effects as the rate of ageing and disease resistance in human and animals. The most important step in preventing oxidative damage and balancing antioxidant defence in the animal body can be to enhance the antioxidant capacity by optimising the dietary intake of antioxidants.

Animal feeds contain a range of different compounds that possess antioxidant activities including vitamin E, consisting of 8 compounds (4 tocopherols and 4 tocotrienols), carotenoids (more than 600 compounds), flavonoids (more than 8,000 compounds), ascorbic acid and some other compounds.

The need for antioxidant defence

Free radicals are typically atoms containing one or more unpaired electrons. Most biologically-relevant free radicals are derived from oxygen and nitrogen, the so-called reactive oxygen species (ROS) and reactive nitrogen species (RNS). Both these elements are essential, but in certain circumstances are converted into free radicals which are highly unstable and their reactive capacity makes them capable of damaging biologically relevant molecules such as DNA, proteins, lipids or carbohydrates (Surai, 2002). The animal's body is under constant attack from free radicals, formed as a natural consequence of the body's normal metabolic activity and as part of the immune system's strategy for destroying invading micro-organisms. 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. 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 ROS per cell per day (Chance et al., 1979). Furthermore Helbock et al. (1998) have shown that the DNA in each cell of a rat

is hit by about 100,000 free radicals a day and each cell sustains as many as 10,000 potentially mutagenic (if unrepaired) lesions per day arising from endogenous sources of DNA damage (Ames and Gold, 1997). If oxidative lesions are not repaired and the basic level of oxidative lesions increased with age, then an old rat can accumulate approximately 66,000 oxidative DNA lesions per cell (Ames, 2003). It is interesting that free radicals also work as physiological mediators and signalling molecules; therefore complete removal of free radicals from the cell could have detrimental consequences.In the case of the immune system, the problem is exacerbated as immune cells produce free radicals and use them to destroy pathogens (Surai, 2002). High oxygen concentration is potentially toxic for living organisms, as first described in laboratory animals in 1878 (Knight, 1998).

Living organisms have evolved specific antioxidant protective mechanisms to deal with free radicals which are constantly produced in the cells. These mechanisms helped them to survive in an atmosphere when oxygen concentration was rising millennia ago, and are described by the general term "antioxidant system" (Surai, 2002). In nature there are thousands of compounds possessing antioxidant properties and able to neutralise free radicals. They can be fat-soluble (vitamin E and carotenoids, coenzyme Q, etc.) and water-soluble (ascorbic acid, glutathione, bilirubin, taurine, etc.), or synthesised in the body (ascorbic acid, glutathione or taurine) or have to be delivered via feed (minerals, vitamin E, carotenoids). More importantly, there is a range of mineral-dependent antioxidant enzymes, which can be synthesised in the body and are able to effectively deal with free radicals, but requires feed-derived mineral co-factors to do so. For example, Se in the form of selenocysteine is an essential part of a family of enzymes called glutathione peroxidases (GSH-Px) and thioredoxin reductases (TR), Zn, Cu and Mn are integral parts of another antioxidant enzyme family called superoxide dismutases (SOD) and iron is an essential part of the antioxidant enzyme called catalase. Therefore only when these metals are delivered with diet in sufficient amounts animal body can synthesise the antioxidant enzymes. In contrast deficiency of those elements causes oxidative stress and damages to biological molecules and membranes.

How antioxidants work

Biological antioxidants react with free radicals or precursor metabolites converting them into less reactive molecules and preventing or delaying oxidation of biological molecules. The most important and well-characterised natural antioxidants in the animal body are vitamins E and C. Plant pigments known as carotenoids have antioxidant capacity. The protective antioxidant compounds are located in organelles, subcellular compartments or the extracellular space enabling maximum cellular protection to occur.

In fact in the body, all antioxidants are working in concert as a team, the "antioxidant system", responsible for prevention of the damaging effects of free radicals and toxic products of their metabolism. In this team, every member has its own job to do and they are located in various parts of the cell in such a way to provide a maximal efficiency of antioxidant protection**.** For example, vitamin E is "headquarter", carotenoid are "communicating services", vitamin C compounds are "special forces" and Se is "chief-executive" of antioxidant defence. The antioxidant 'team' acts to control levels of free radical formation, as a coordinated system where deficiencies in one component impacts the efficiency of others. For example, ascorbate can help vitamin E to recover from oxidation to become active again. If relationships in this team are effective, which happens only in the case where the individual has a balanced diet and sufficient intake of antioxidants, then even low doses of such antioxidants as

vitamin E or Selenium can be effective. 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, especially minerals such as 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 list of possible stresses in poultry production includes the following (Surai, 2002; 2006):

- *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 Se concentration in combination with other antioxidants (vitamin E and carotenoids) 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.
- *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.
- *Sub-optimal temperatures in the poultry house.* Cold tolerance as well as feather cover is influenced by thyroid hormone activity, which is Se-dependent.

- *High levels of ammonia and CO_2 in poultry house as a result of inadequate ventilation* can 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 membranes and hence, important organelles, 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 on its antioxidant functions.
- *Vaccination* is a substantial stress; and in some cases using vitamin E as a vaccine adjuvant can help improve vaccination efficiency.
- *Induced molting with feed withdrawal* is an important stress condition when decreased efficiency of heterophil function increases bird susceptibility to various infections.
- *Mycotoxins in the feed* can decrease antioxidant assimilation from the feed and increase their requirement to prevent damaging effects of free radicals and toxic products of mycotoxin metabolites. It is now recognised that at least 25% of world's grains are contaminated with mycotoxins.
- *Heavy metals and other toxicants (dioxine, pesticides, fungicides, herbicides, etc.) in the feed* can also cause an oxidative stress, decreasing immunocompetence, productive and reproductive performances and increasing a requirement for antioxidants.
- *Oxidized fat in the diet* increases the exposure to free radicals, inducing stress in the intestine and increasing antioxidant requirement to prevent damage to tissues. When a chicken diet includes fat, which has undergone high temperature treatment, resulting peroxides can contribute substantially to oxidative stress.
- *Extensive preventive medication (coccidiostats or other veterinary drugs in the diet)* can limit dietary antioxidant uptake or increase stress conditions, e.g. monensin can stimulate lipid peroxidation in the chicken liver. Similarly, oral furazolidone treatment of chickens has been associated with a decreased vitamin E concentration and increased lipid peroxidation in their liver.
- *Vitamin A excess* in the diet has been shown to cause 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.

Practical applications of natural antioxidants in poultry nutrition

Natural antioxidants and their correct application, in terms of forms used and delivery systems exert a major influence on poultry production, in particular on poultry reproduction.

Effect of natural antioxidants on fertility

Chicken spermatozoa are unique in their structure and chemical composition. The most important feature of lipid composition of the avian semen is the extremely high proportions of long–chain polyunsaturated fatty acids (PUFAs) in the phospholipid fraction of spermatozoa. On the one hand, the high PUFA proportion is a necessity in order to maintain specific membrane properties (fluidity, flexibility, etc). On the other hand, spermatozoa became very susceptible to lipid peroxidation and, therefore, the antioxidant defence is considered to be a key element in maintaining semen quality. Vitamin E was discovered as a "vitamin of reproduction" in 1922. In recent years it has been shown that vitamin E is located in spermatozoa and provides antioxidant protection, especially in stress-conditions of in vitro semen manipulation, including dilution, storage and deep freezing of spermatozoa (see Surai, 2002 for a review). Furthermore, it was shown that vitamin E provides additional protection in the case of fatty acid manipulation of the semen (Surai et al., 2000; Zinini et al., 2003; Cerolini et al., 2005). However, in some studies the vitamin E dose-response in cockerels was shown to be non-linear (Lin et al., 2005).

Se supplementation is known to affect the antioxidant defenses of chicken semen (Surai et al., 1998). Furthermore, Edens (2002) showed that, when cockerels were fed on a basal diet containing 0.28 ppm Se without additional dietary supplementation of this trace element, the percentage of normal spermatozoa was only 57.9% and two major abnormalities seen were bent midpiece (18.7%) and corkscrew head (15.4%). When this diet was supplemented with an additional 0.2 ppm Se in the form of selenite, the percentage of normal spermatozoa increased to 89.4% and abnormalities in the form of bent midpiece and corkscrew head were decreased down to 6.2 and 1.8% respectively. However, when organic Se was included in the cockerel's diet in the same amount, semen quality was further improved and those abnormalities decreased down to 0.7 and 0.2% and the percentage of normal spermatozoa increased up to 98.7%. These results clearly showed that the form of dietary Se supplementation is a crucial factor of its efficiency, with organic Se being much more effective in comparison to selenite. Therefore Se deficiency is associated with midpiece damage to spermatozoa It is clear that the midpiece of spermatozoa of the Se-deficient male is broken. In such conditions, sperm motility and fertilizing capacity would be compromised. Organic selenium can also improve fertility and, more importantly, increase the duration of fertility (Agate *et al*., 2000). Preliminary observations in female chickens have also revealed the effectiveness of dietary supplementation with vitamin E, organic Se or both, to sustain fertility in aging flocks (Breque *et al*., 2003). Thus avian spermatozoa might be expected to have systems which will maintain stability throughout this period. Indeed, recent results have confirmed the existence of a complex antioxidant system in the utero-vaginal portion of the fowl oviduct (Breque and Brillard, 2002). In particular GSH-Px activity in the utero-vaginal junction was 12-fold higher than in the liver.

It seems likely, that organic Se at 0.3 ppm could also be effective in male turkeys. In one study, sodium selenite (0.3 ppm) was replaced by organic Se (Sel-Plex) in the diet of male turkeys (Dimitrov *et al*., 2007). After 30 weeks of feeding, the semen samples were collected and analyzed. After 6 hours of semen storage, motility decreased in the control group by 8.7%, while in semen from Sel-Plex-supplemented toms motility decreased much less (by 3.95%). The fertilizing ability of stored semen was 88% in the control group and 90.5% in the Sel-Plex-supplemented group.

Effect of natural antioxidants on hatchability

The hatching process is considered to be a time of oxidative stress. Therefore, improved antioxidant defences during embryonic development potentially could increase hatchability. It was shown that vitamin E, carotenoids and Se can be transferred from the diet to the egg and consequently to the developing embryo (Surai, 2002). There are species-specific differences in vitamin E transfer from feed to the egg and, therefore, to the developing embryo. In particular, when chicken, turkey, goose and duck diets were supplemented with the same amount of vitamin E, the highest alpha-tocopherol concentration was found in chicken eggs and chicken embryonic tissues (Surai et al., 1998). Increased vitamin E concentration in the chicken embryonic tissues was associated with decreased tissue susceptibility to lipid peroxidation (Surai et al., 1999). Similarly, increased carotenoid concentration in the chicken embryo decreased the susceptibility of the tissues to lipid peroxidation (Surai and Speake, 1998; Surai et al., 2003). It seems likely that protective effect of vitamin E can be seen more clearly in stress condition, for example, in laying hens consuming T-2 toxin (Tobias et al., 1992) or in birds that consume vicine (from faba beans) (Muduili et al., 1982).

Se transfer from the diet to the egg and then on to the developing embryo has, so far, received only limited attention. However, it is proven that it is only organic Se that is effectively transferred, since the major form of Se in the egg is SeMet, but the chicken cannot synthesise this compound. Our data indicate that Se in the maternal diet affects Se concentration in tissues of the postnatal quail. Indeed, when newly hatched quail from Se-enriched eggs and normal quail eggs were placed on low Se-diet (0.1 ppm), the Se concentration in tissues dropped dramatically for the first 2 weeks posthatch (Surai et al., 2006). This finding suggests that Se in the liver of newly hatched quail is rapidly diluted by the post hatch growth of the tissue unless the diet of the neonate is supplemented with this element. It is possible to suggest that Se absorption from the diet is not optimal during the first few days of life and the chick must rely on reserves of the element accumulated during embryogenesis. However, the difference in tissue Se concentrations between the control and experimental groups was still significant at 2 weeks posthatch. These results clearly indicate that the maternal diet affects not only newly hatched quails, but also has a sustained effect on the chicks during postnatal development.

Recently a new experiment was conducted at SAC to address this question (Pappas et al. 2005). The maternal diet was supplemented with 0.4 ppm organic Se in the form of Sel-Plex and comparison was made with a basic diet containing 0.1 ppm feed-derived Se. As a result of dietary Se supplementation the Se concentration in the egg yolk, albumin, shell, shell membrane and perivitelline membrane was significantly increased. The newly hatched chicks were placed on a basal diet (0.1 ppm) without Se supplementation for the next 4 weeks posthatch. After hatching, chickens fed diets low in Se (0.1 ppm), but originating from parents fed diets high in Se (0.5 ppm), had, up to 4 weeks post-hatch, significantly higher blood Se levels than those that originated from parents fed diets low in Se (0.1 ppm). It seems likely that Se inadequacy is more often observed in commercial breeders, since Se supplementation in the organic form is shown to positively affect hatchability (Sefton and Edens, 2004; Edens and Sefton, 2003; Sefton and Edens, 2004a; Renema and Sefton, 2004; Renema, 2004; Renema. 2003).

References

Agate, D.D., O'Dea, E.E. and Rustad M.E. (2000). Effects of dietary selenium on laying hen fertility as assessed by the periviteline sperm hole assay Proc Poultry Research and Production Symposium, Alberta Poultry Research Centre, pp. 1-4.

Ames, B.N. (2003) An Enthusiasm for Metabolism. *J. Biol. Chem.* **278**: 4369-4380.

Ames, B.N. and Gold, L.S. (1997). The causes and prevention of cancer: gaining perspective. *Environ. Health. Perspect.* **105** Suppl 4: 865-873.

Breque, C. and Brillard, J.P. (2002). Sperm storage in the avian oviduct: baselines for a complex antioxidant system in the sperm storage tubules. *Archiv Geflugelkunde* **66**: 83.

Breque, C., Surai, P.F. and Brillard J.P. (2003). Roles of antioxidants on prolonged storage of avian spermatozoa *in vivo* and *in vitro*. *Mol. Reprod. Develop.* **66**: 314-323.

Cerolini S, Surai PF, Speake BK, Sparks NH. (2005). Dietary fish and evening primrose oil with vitamin E effects on semen variables in cockerels. *Br Poult. Sci.* **46**: 214-222.

Chance, B., Sies, H. and Boveris, A. (1979). Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* **59**: 527-605.

]Dimitrov, S.G., Atanasov, V.K., Surai, P.F. and Denev, SA. (2007). Effect of organic selenium on Turkey semen quality during liquid storage. *Anim. Reprod. Sci*. **100**: 311-317.

Edens, F.W. (2002). In: Nutritional Biotechnology in the Feed and Food industries. Proceedings of 18th Alltech's Annual Symposium, Edited by Lyons, T.P. and. Jacques, K.A., Nottingham University Press, Nottingham, UK, pp. 29-42.

Edens, F.W. and Sefton, A.E. (2003). Poster presented at Alltech's 19th Annual Symposium on Nutritional Biotechnology in the Feed and Food Industries, Lexington, Ky, May 12-14, 2003, CD-ROM.

Helbock, H.J., Beckman, K.B., Shigenaga, M.K., Walter, P.B., Woodall, A.A., Yeo, H.C. and Ames, B.N. (1998). DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. *Proc. Natl. Acad. Sci*. **95**: 288-293.

Knight, J.A. (1998). Free radicals: Their history and current status in aging and disease. *Annals of the Clinical and Laboratory Sciences* **28**: 331-346.

Lin YF, Chang SJ, Yang JR, Lee YP, Hsu AL. (2005). Effects of supplemental vitamin E during the mature period on the reproduction performance of Taiwan Native Chicken cockerels. *Br. Poult. Sci.* **46:** 366-373.

Muduuli, D.S., Marquardt, R.R. and Guenter, W. (1982). Effect of dietary vicine and vitamin E supplementation on the productive performance of growing and laying chickens. *Br. J. Nutr.* **47**: 53-60.

Pappas, A.C., Karadas, F., Surai, P.F. and Speake, B.K. (2005). The selenium intake of the female chicken influences the selenium status of her progeny. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* **142:** 465-474.

Renema, R.A. (2003). Abstracts of the 92nd Annual Meeting of the Poultry Science Association. July 6-9, 2003, Madison, Wisconsin, USA. *Poultry Science* **82** (Suppl. 1): 51.

Renema, R.A. (2004). In: Bnutritional Biotechnology in the Feed and Food Industries. Proceedings of 20th Alltech's Annual Symposium, Edited by Lyons, T.P. and. Jacques, K.A., Nottingham University Press, Nottingham, UK, pp. 81-91.

Renema, R.A. and Sefton, A.E. (2004). Book of Abstracts XXII World's Poultry Congress, 8-13 June, 2004, Istanbul, Turkey, p.521.

Sefton, A.E. and Edens, F.W. (2004) In: Biotechnology in the Feed and Food Industry. Proceedings of the 20th Annual Symposium (Suppl. 1), May 22-26, 2004, Lexington, Kentucky, USA, p. 33.

Sefton, A.E. and Edens, F.W. (2004a).In: Nutritional Biotechnology in the Feed and Food Industry. Proceedings of the 20th Annual Symposium (Suppl. 1), May 22-26, 2004, Lexington, Kentucky, USA, p. 32.

Surai, P.F., Kostjuk, I., Wishart, G., MacPherson, A., speake, B.K., Noble, R.C., Ionov, I.A. and Kutz, E. (1998). Effect of vitamin E and selenium of cockerel diets on glutathione peroxidase activity and lipid peroxidation susceptibility in sperm, testes and liver. Biol. Trace Elem. Res. 64: 119-132

Surai, P.F., Noble, R.C., Sparks, N.H.C. and Speake, B.K. (2000). Effect of long-term supplementation with arachidonic or docosahexaenoic acids on sperm production in the broiler chicken. *J. Reprod. Fertil.* **120**: 257-264.

Surai, P.F. (2002). *Natural Antioxidants in Avian Nutrition and Reproduction*. Nottingham University Press, Nottingham.

Surai, P.F. (2006). Selenium in Nutrition and Health. Nottingham University Press, Nottingham.

Surai, P., Ionov, I., Kuchmistova, E., Noble, R. and Speake, B. (1998). The relationship between the levels of α-tocopherol and carotenoids in the maternal feed, yolk and neonatal tissues: Comparison between the chicken, turkey, duck and goose *J. Sci. Food Agric.* **76:** 593-598.

Surai, P.F., Noble, R.C. and Speake, B.K. (1999). Relationship between vitamin E content and susceptibility to lipid peroxidation in tissues of the newly hatched chick. *Brit. Poultry Sci.* **40**: 406-410.

Surai, P.F. and Speake, B.K. (1998). Distribution of carotenoids from the yolk to the tissues of the chick embryo. *J. Nutr. Biochem.* **9**: 645-651.

Surai, A.P., Surai, P.F., Steinberg, W., Wakeman, W.G., Speake, B.K. and Sparks, N.H.C. (2003). Effect of canthaxanthin content of the maternal diet on the antioxidant system of the developing chick. *Brit. Poultry Sci.* **44**: 612-619.

Surai PF, Karadas F, Pappas AC, Sparks NH. (2006). Effect of organic selenium in quail diet on its accumulation in tissues and transfer to the progeny. *Br. Poult. Sci.* 47: 65-72.

Tobias, S., Rajic, I. and Vanyi, A. (1992). Effect of T-2 toxin on egg production and hatchability in laying hens. *Acta Vet. Hung.* **40**: 47-54.

Zanini, S.F., Torres, C.A., Bragagnolo, N., Turatti, J.M., Silva, M.G. and Zanini, M.S. (2003). Evaluation of the ratio of omega6: omega3 fatty acids and vitamin E levels in the diet on the reproductive performance of cockerels. *Arch. Tierernahr.* **57:** 429-442.