THE RELEVANCE OF THE ANTIOXIDANT SYSTEM TO THE HEALTH AND GROWTH OF THE DEVELOPING CHICK

P.F. SURAI

It is generally accepted that living organisms pay a price for living in the oxygen atmosphere. Although oxygen is absolutely necessary to maintain energy production in living organisms, when in excess it is toxic. Information is accumulating to indicate that free radicals and other reactive oxygen species are produced in the metabolic pathways of aerobic cells and affect a number of biological processes (Halliwell, 1994). Furthermore, the electron transport chain of the mitochondria is considered to be the main source of free radicals (mainly as superoxide radical) in biological systems. Other sources of free radicals include immune system cells (macrophages) and enzymes of xenobiotic metabolism. Free radicals and toxic products of their metabolism have been postulated to play a role in aging and are implicated in a number of degenerative diseases (Hogg, 1998). In fact, it is believed that most human diseases at different stages of their progress are associated with free-radical-mediated processes. Furthermore free radicals are considered to play a role as subcellular messengers in gene regulatory and signal transductory pathways (Sen, 2000). Redox regulation of gene expression by superoxide and other related oxidants and antioxidants is beginning to unfold as a vital mechanism in health and disease (McCord, 2000). Unfortunately, this subject is much less studied in relation to animals but information is also accumulating which shows the role of free radicals in animal production. In particular, in the poultry industry such diseases as Pulmonary Hypertension Syndrome (PHS) (Bottje and Wideman, 1995), nutritional muscular dystrophy, encephalomalacia, exudative diathesis (Combs, 1994) and some others are associated with overproduction of free radicals. Therefore, antioxidants could play an important role in their prevention.

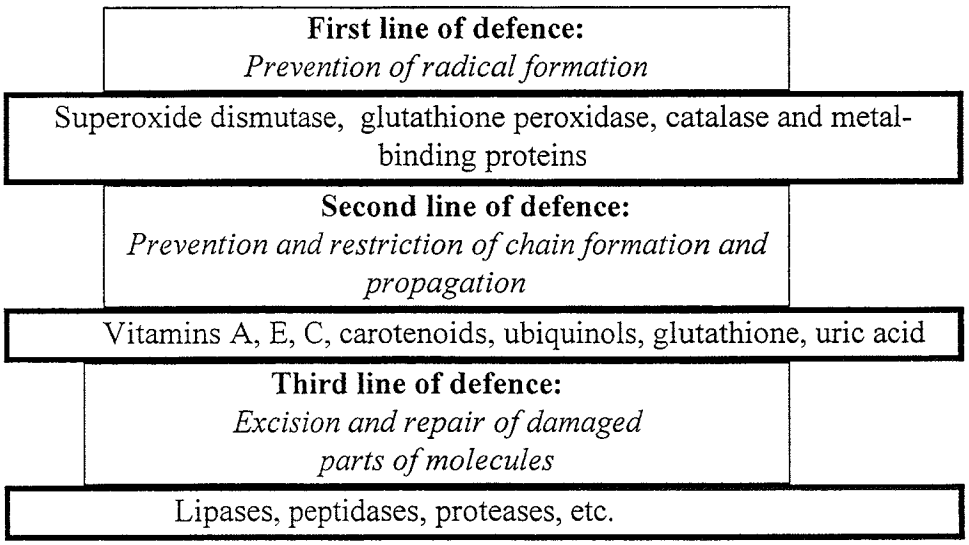

Figure 1. Three lines of defence in animal cells.

Avian Science Research Centre, SAC, Auchincruive, Ayr, UK.

During evolution living organisms have developed specific antioxidant protective mechanisms to deal with the free radicals which are constantly produced in the cells. These mechanisms are described by the general term "antioxidant systems". They are diverse and are responsible for the protection of cells from the actions of free radicals. They include natural fat-soluble antioxidants (vitamins A, E, carotenoids and ubiquinones), water-soluble antioxidants (ascorbic acid, glutathione and uric acid) and antioxidant enzymes: glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) 1999; Figure1). The protective antioxidant compounds are located in organelles, subcellular compartments or the extracellular space enabling maximum cellular protection to occur. Therefore, all the antioxidants in the cell operate in association with each other forming integrated antioxidant systems. The cooperative interaction between antioxidants in the cell is vital for maximum protection from the deleterious effects of free radicals. It has been suggested that the antioxidant-prooxidant balance is a major determinant of successful chick embryo development and of early postnatal development (Surai, 1999).

This assumption is based on the following characteristics of the newly hatched chick.

(a) High levels of polyunsaturated fatty acids (PUFA)

The newly hatched chick is considered to be an intermediate stage between prenatal and postnatal development. In this respect avian embryo development is associated with the accumulation of highly polyunsaturated lipids within the tissues (Noble and Speake, 1997). Also, the rate of oxidative metabolism increases dramatically over the hatching period (Freeman and Vince, 1974). The hatching process itself can be considered as a high stress condition which the newly hatched chicken has to accommodate. Thus, effective antioxidant protection may be vital for posthatch viability and subsequent productive and reproductive performances. In such conditions, oxidative stress may be a problem during the last days of prenatal and the first days of postnatal chick life. These necessitate the development of effective antioxidant capacities in the tissues to prevent lipid peroxidation.

The tissues of the newly hatched chick show distinctive features in antioxidant profile and susceptibility to lipid peroxidation. The susceptibility of tissues to peroxidation depends on a number of factors including primarily the content of PUFA, levels of natural antioxidants (vitamins A, E, C and carotenoids), activities of antioxidant enzymes (SOD, GSH-Px and CAT), their cofactors (Se, Zn and Mn) and content and availability of prooxidant cations (Fe and Cu).

The brain clearly displays the greatest susceptibility to spontaneous and Fe-stimulated lipid peroxidation. High levels of lipid unsaturation and comparatively low antioxidant protection make the brain vulnerable to free radical attack. This is of particular importance in the chick in respect to the development of encephalomalacia which is associated with an antioxidant system compromised by a deficiency of vitamin E (Fuhrmann and Sallmann, 1995). In such conditions the cerebellum was shown to display particular oxidative stress. Packer and Landvik (1989) suggested that in biological systems vitamin E can be recycled from its oxidised form, ascorbic acid being one of the possible antioxidants involved in such a recycling (Packer, 1992). In the embryonic brain it has been suggested that antioxidant defence is afforded by high levels of ascorbic acid which is able to effectively recycle the low concentrations of vitamin E and maintain membrane protection against free radical attack (Surai *et al.*, 1996).

The liver is the main site of natural antioxidant accumulation and metabolism. Vitamin E and carotenoid accumulation in the liver reached a maximum at hatching (Surai *et al.*, 1996)

and is accompanied by high activities of GSH-Px and CAT (Table 1). Thus, the susceptibility of the liver lipids to peroxidation in the newly hatched chick immediately following hatching is low. The high levels of endogenous antioxidants within the liver can clearly serve as a major adaptive mechanism for the protection of the tissue during the oxidative stress experienced at hatching. Carotenoids can also be considered as a group of natural antioxidants of some importance for avian embryo development (Surai and Speake, 1998). Lutein and zeaxanthin are characterised by high antioxidant activity (Rice-Evans *et al.*, 1997) and are the main carotenoids accumulated in high concentration in the liver, but in much lower concentrations in other tissues. In general, carotenoid accumulation in the chicken embryo occurs in a similar manner to that of vitamin E, reaching a maximum level at hatching (Surai and Speake, 1998). Within the liver of the newly hatched chick it has been shown that the massive accumulation of lipids occurs through the formation of droplets within the cytosol (Noble and Cocchi, 1990) thus providing in turn a suitable intracellular milieu for the storage of large amounts of lipid-soluble vitamins.

Table 1. Antioxidant and fatty acid composition of tissues of a newly hatched chick (Adapted from Surai *et al.*,1999b).

	Liver	Brain	Kidney	Heart	Lung	Muscle
Vitamin E, μg/g	678.2	6.6	19.5	24.3	21.0	14.8
Vitamin A, μg/g	10.3	0.1	1.3	0.4	-	0.1
Carotenoids, μg/g	30.8	-	2.3	3.3	2.7	2.0
Ascorbic acid, μg/g	151.0	839.1	130.6	59.0	124.6	57.9
Glutathione, μg/g	43.3	40.2	54.7	32.9	23.6	21.3
Mn-SOD, U/mg protein	3.8	3.7	3.0	5.8	0.1	1.1
Cu-Zn-SOD, U/mg protein	1.5	6.9	3.1	2.7	5.8	6.1
Se-GSH-Px, U/mg protein	177.0	29.8	159.8	99.0	99.8	45.8
Non-Se-GSH-Px, U/mg protein	114.6	6.2	58.6	11.6	53.0	12.6
Catalase, U/mg protein	35.8	1.9	29.5	5.8	6.0	3.2
18:2n-6, %[1]	12.3	1.5	11.8	9.8	8.5	15.0
20:4n-6, %[1]	21.5	9.5	19.9	21.8	10.1	15.3
22:6n-3, %[1]	10.1	16.2	6.3	3.3	3.3	8.1

[1] PUFAs in the phospholipid fraction.

The importance of the heart-vascular system for posthatch development determines the strategy of antioxidant defence. Data (Surai *et al.*, 1999b) has shown that the heart is characterised by moderate levels of both fat- and water-soluble antioxidants. Most significantly, the activity of Mn-SOD in the heart was much higher than that in the other tissues. With the considerable increase in the activity of the heart mitochondria associated with the hatching process leakage of electrons from the electron-transport chain has been suggested to be the main source of superoxide radicals in this tissue (Turrens, 1997). The SOD forms the first line of defence against free radical damage and, thus, the high activity of the mitochondrial SOD is possibly of vital importance to the heart at the critical period of hatching.

The requirement in antioxidant/prooxidant balance in the lung is exemplified by its involvement in the development of PHS in the chicken (Bottje and Wideman, 1995).

However, the lung also displayed a very high concentration of Fe and the enhanced susceptibility of the lung to spontaneous lipid peroxidation compared to other tissues may be associated with such high concentration of Fe. Nevertheless, it is clear that the lung possesses considerable protection against peroxidation through its content of Cu,Zn-SOD, Se-GSH-Px and Non-Se-GSH-Px.

As in the case of the brain the high levels of PUFAs in the muscle made the tissue vulnerable to lipid peroxidation, especially in the presence of catalytic amounts of Fe. With only low levels of fat-soluble antioxidants, comparatively low levels of Se, reduced glutathione and ascorbic acid, the muscle presumably relies upon its high levels of Cu,Zn-SOD for antioxidant protection. In the case of the chick embryo there is evidence that in conditions under which vitamin E concentrations are compromised, the levels of Cu,Zn-SOD are unable to afford adequate protection with the result that at hatching exudative diathesis with muscular degeneration can be observed (Hassan *et al.*, 1990).

A highly significant correlation between the Se level and the activity of Se-GSH-Px was found in the majority of the tissues (Surai *et al.*, 1999b). A carry over effect of Se from the maternal diet via the chicks has been shown (Hassan *et al.*, 1990; Surai, 2000) with an associated increase in the activity of GSH-Px and reduction in exudative diathesis. It has been suggested that the effect of Se on the activity of GSH-Px is achieved through pretranslational mechanisms including Se-GSH-Px gene expression and cytosolic mRNA stabilisation (Christinsen and Burgener, 1992).

Thus, tissue-specific distinctive features associated with the level of PUFA, antioxidant enzyme activity, natural antioxidant accumulation and the susceptibility to lipid peroxidation have been shown (Surai *et al.*, 1999b). In another investigation (Surai, 1999a) it was shown that different tissues of the chick embryo displayed distinct developmental strategies with regard to the acquisition of antioxidant capacity and it was suggested that during embryogenesis natural antioxidants (vitamins A, E, C and carotenoids) play a crucial role in antioxidant defence of the embryo tissues against lipid peroxidation. However, it may also be concluded that in postnatal development, when oxygen concentrations in the tissues are higher, metabolic activity and superoxide radical production are increased and tocopherol and carotenoid concentrations are decreased, the required protection is afforded through the major antioxidant enzymes SOD, GSH-Px and CAT. In fact, recent observations (Surai, 2000) indicate that GSH-Px activity in the chicken liver significantly increased between hatch and 10 days of postnatal development suggesting the different strategy in antioxidant system regulation. Indeed, during embryonic development, vitamin E played a key role in antioxidant defence, but after hatching its level in the liver decreased quickly (more than 10 times during the first 10 d) while simultaneously the activity of GSH-Px was increasing. It seems that the antioxidant systems of the growing chick rely more on GSH-Px as a major defence. Therefore, dietary provision of physiological levels of selenium is an essential step in maintaining antioxidant defence.

(b) Immune system development

In the newly hatched chick the immune system is immature and is not completely functional. This system is actively developing in postnatal life and this process involves accumulation of PUFAs and increased susceptibility to lipid peroxidation. Furthermore, immune cells use free radicals as an effective weapon to kill pathogens (Kettle and Winterbourn, 1997) and, as a result, the surrounding tissues could be damaged if antioxidant protection is not appropriate (Schwarz, 1996). In stress conditions the requirement for

antioxidant defence substantially increased. For example, under commercial conditions, chicks may be delayed access to feed for a considerable time after hatch and this increases the likelihood of ketosis and dehydration (Vieira and Moran, 1999). The delays between emergence and access to food and water resulted from the time spent in the hatchery and transportation to the farm and could be considered as substantial stress conditions. The asynchrony of chick emergence (eggs from older breeders and smaller eggs tend to hatch earlier) from the egg means that chicks hatching early may be held 36 h longer than those hatching late (Vieira and Moran, 1999). This could also result in dehydration and a shortage of available energy and lead to subsequent reduction in the rate of nutrient absorption and growth rate and increased early mortality. Immune system development is also compromised due to this stress (Wyatt *et al.*, 1986; Casteel *et al.*, 1994). Therefore, during these extreme stress conditions the antioxidant system could be the crucial factor in maintaining chicken health. It is necessary to underline the fact that any birds with restricted nutrition immediately after hatching are not able to recover completely and do not reach the same weight gain as those that are fed early (Vieira and Moran, 1999). In the absence of growth promoters in the chicken feed, the role of natural antioxidants in immune system modulation is difficult to overestimate.

(c) Digestive system development

The contents of the yolk sac are the main source of nutrients during the first 2-3 d after hatching and after this time the main energy source for the chick changes from yolk-based lipid to dietary carbohydrate (Vieira and Moran, 1999). The digestive and absorptive activities of the intestine actively develop during the first 2 weeks posthatch (Vieira and Moran, 1999) and this development is also associated with PUFA metabolism and possible lipid peroxidation. At the same time the ability to utilise carbohydrates is developing (Siddons, 1969) and by 4 d after hatch the ability to digest starch can reach 85% (Noy and Sklan, 1995). In contrast, pancreatic lipase activity increases until 16 d after hatching (Vieira and Moran, 1999). The intestine attains maximum growth between 3 and 7 d posthatch (Murakami *et al.*, 1992). In addition, early access to food stimulates the growth of the intestines and their absorptive capacity (Moran, 1985). On the other hand, a delay in access to food and absence of stimulation from food intake causes shortening and thinning of the villi (Michael and Hodges, 1973; Vieira and Moran, 1999) which results in decreasing absorptive efficiency of the intestine.

It is generally accepted that the ability to absorb dietary lipids is not well developed in the newly hatched chick but improves with age and it is recommended to avoid long chain saturated fatty acids and to use unsaturated fat during at least the first week posthatch (Vieira and Moran, 1999; Noy and Sklan, 1995). It is obvious that vitamin E is poorly assimilated from the diet during this period (at least during first 5 d posthatch) when it is extremely important as a natural antioxidant and as a result the chicken actively uses tocopherol reserves accumulated in the liver during embryonic development.

For example, during early postnatal life the level of tocopherols in the turkey liver have been observed to decrease sharply (Surai *et al.*, 1998; Soto-Salanova and Sell, 1995). Therefore the reserve of vitamin E is used during the first 2 weeks posthatch. During this period vitamin E concentration in the liver decreased by 10 times in chickens (Surai and Ionov, 1994), goslings and ducklings (Surai *et al.*, 1993) and more than 50 times in turkeys (Soto-Salanova *et al.*, 1993). On the other hand, during this period of development, tissues with incompletely developed antioxidant regulation required an effective protection against

lipid peroxidation. These data also confirmed the biological importance of a very high vitamin E concentration in the embryonic liver at hatching time.

A variety of approaches aimed at improving the vitamin E status of turkey poults has been investigated, including dietary supplementation of the poults with high levels of vitamin E (Applegate and Sell, 1996), bile salts (Soto-Salanova *et al.*, 1993) and fat (Soto-Salanova and Sell, 1995) as well as vitamin E injection (Soto-Salanova and Sell, 1996) and alterations in dietary fatty acid profile (Applegete and Sell, 1996). However, none of these attempts were able to reverse the process and only slowed down the vitamin E depletion. Nevertheless, observations with chickens indicate that increased vitamin E supplementation, as well as inclusion of organic selenium in the maternal diet, could substantially improve the situation (Surai, 2000). Therefore, maternal diet plays a crucial role in maintaining physiological levels of vitamin E in chicken tissues during the first 10 d of postnatal development.

(d) Antioxidant system regulation in embryonic and postnatal development of the chicken

Antioxidant transfer from the maternal diet to the egg yolk and further to the developing chick could be considered as a valuable means of regulating the antioxidant system of the newly hatched chick. Vitamin E transfer from the yolk to the developing tissues of the chick takes place during the last week of incubation, reaching a maximum in the newly hatched chick (Surai *et al.*, 1996). There also are species, specific differences in vitamin E and carotenoid transfer from the feed to the egg yolk and further to the developing embryonic tissues and chickens seem to have a better ability to accumulate these nutrients compared to turkeys, ducks or geese (Surai *et al.*, 1998). There is also discrimination between tocopherols and tocotrienols during embryonic development and alpha-tocopherol is the major vitamin E form in chicken tissues (Surai and Speake, 1998a).

Vitamin E transfer from the feed to the egg yolk is a fast process (Surai *et al.*, 1999) but there are no real reserves of vitamin E in the body of the laying hen and, therefore, with a single egg more vitamin E is released than the total amount present in the liver (Surai *et al.*, 1998a). Therefore, the vitamin E level in the diet is extremely important in maintaining physiological levels of this vitamin in the yolk. Increased vitamin E supplementation of the maternal diet significantly increased the vitamin E level in the egg yolk and embryonic tissues (Surai *et al.*, 1999a) and, as a result, the liver of newly hatched chicks became more resistant to lipid peroxidation. Similarly, carotenoid supplementation of the maternal diet was also associated with increased carotenoid accumulation in the egg yolk and the developing tissues (Surai and Speake, 1998). The carotenoid-enriched egg yolk, yolk sac membrane and liver of the newly hatched chick was characterised by a decreased susceptibility to lipid peroxidation.

Inclusion of organic selenium in the maternal diet is an effective means of increasing the selenium concentration in the egg yolk and egg white. A combination of organic selenium with increased vitamin E supplementation did not change selenium accumulation in the egg. Increased selenium level in the egg yolk and egg white resulted in an increased selenium concentration in the liver of the newly hatched chick. As a result the activity of GSH-Px significantly increased (Table 2).

Organic selenium had also a positive effect on vitamin E accumulation in the egg yolk and embryonic liver as well as increased accumulation of reduce glutathione in the liver. The increased antioxidant levels in the chick liver as a result of organic selenium supplementation translated into increased tissue resistance to lipid peroxidation. The most striking feature of the effect of selenium in the maternal diet on the antioxidant systems of the developing chick is that it was still obvious 5 d posthatch and, in the case of the combination of vitamin E and

selenium in the maternal diet, the beneficial effect was still seen at 10 d of postnatal development: vitamin E level increased and lipid peroxidation declined.

Table 2. Effect of organic selenium on the antioxidant status of the egg and liver of a newly hatched chick[1] (Adapted from Surai, 2000).

Parameter	Diet		
	Commercial (C)	C+ 0.2 mg/kg Se	C+ 0.4 mg/kg Se
Se in egg yolk, ng/g	298.3	605.3	854.0
Se in albumin, ng/g	50.7	193.7	403.7
Vit. E in egg yolk, μg/g	19.6	32.2	45.5
Vit. A in egg yolk, μg/g	6.3	6.0	6.1
Vit. E in liver, μg/g	119.6	144.2	166.1
Vit. E in plasma, μg/g	8.2	9.9	10.2
Vit. E in brain, μg/g	1.5	1.9	1.9
Glutathione in liver, μg/g	482.9	667.6	696.5
Se-GSH-Px, U/g fresh tissue	15.8	24.5	27.1
MDA in liver, μg/g	22.7	16.4	14.2

[1] The level of selenium and vitamin E in the commercial diet was 171 μg/kg and 10.1 mg/kg respectively. Selenium was supplemented in the form of Sel-Plex.

The important point is that effects of dietary antioxidants on the chicken are most pronounced when any stress conditions appear. Indeed, in ideal physiological conditions, when hatchability is higher than 85% and mortality for the first 10 d posthatch is negligible, there is not much scope to improve the situation. However, when stress conditions occur and free radical production exceeds antioxidant protection dietary antioxidant supplementation is especially helpful. This was shown in the case of ascites (Roch *et al.*, 2000) when antioxidant (vitamin E and selenium) supplementation substantially decreased mortality. Similar results were obtained when chickens experienced anaemia and organic selenium supplementation of the maternal diet significantly (more than 3 times) decreased mortality for the first two weeks posthatch (Lanning *et al.*, 2000). Another confirmation of the positive effect of antioxidants for poultry under stress conditions came from studies where very high vitamin E supplementation prevented the decline in egg production due to high temperature (Bollengier-Lee *et al.*, 1998, 1999).

In conclusion, information is actively accumulating to indicate that antioxidant systems are among the major regulators of many physiological processes, and antioxidant/prooxidant balance in the chicken body is responsible for maintaining their health, growth and future productive and reproductive performances. Clearly more work is needed to address this question.

REFERENCES

Applegate, T.J. and Sell, J.L. (1996). *Poultry Science,* **75:** 881-890.

Bollengier-Lee, S., Mitchell, M.A., Utomo, D.B., Williams, P.E. and Whitehead, C.C. (1998). *British Poultry Science,* **39**:106-112.

Bollengier-Lee S., Williams, P.E. and Whitehead, C.C. (1999). *British Poultry Science,* **40:** 102-107.

Bottje, W.G. and Wideman, R.F. (1995). *Poultry and Avian Biology Reviews,* **6**: 211-231.

Casteel, E.T., Wilson, J.L., Buhr, R.J. and Sunder, J.E. (1994). *Poultry Science,* **73**: 1679-1684. Christensen M.J. and Burgener K.W. (1992). *Journal of Nutrition,* **122:** 1620-1626.

Combs, F.F.Jr. (1994). *Veterinary Clinical Nutrition,* **1:** 133-140.

Freeman, B.M. and Vince, M.A.(1974) *Development of the Avian Embryo.* Chapmann and Hall, London, pp. 249-260

Fuhrmann, H. and Sallmann, H.P. (1995). *Annals of Nutrition and Metabolism,* **39:** 302-309.

Halliwell, B. (1994). *Nutrition Reviews*, **52**, 8, 253-265.

Hassan, S., Hakkarainen, J., Jonsson, L. and Tyopponen, J. (1990). *Journal of Veterinary Medicine, A.* **37:** 708-720.

Hogg, N. (1998). *Seminars in Reproductive Endocrinology,* **16**: 241-248.

Kettle, A.J. and Winterbourn, C.C. (1997). *Redox Report,* **3**: 3-15.

Lanning, D., Ayres, K. and Kenyon, S. (2000). Sel-Plex in the breeder diet: reductions in chick mortality: summary of commercial studies in Britain 2000. Alltech UK, Stamford, Lincs. UK.

McCord, J.M. (2000). *American Journal of Medicine,* **108**: 652-659.

Michael, E. and Hodges, R.D. (1973). *Histochemie,* **36**: 39-49.

Moran, E.T. (1985). *Journal of Nutrition,* **115**: 665-674.

Murakami, H., Akiba, Y. and Horiguchi, M. (1992). *Growth, Development and Ageing,* **56**: 75-84.

Noble, R.C. and Cocchi, M. (1990). *Progress in Lipid Research,* **29**: 107-140.

Noble, R.C. and Speake, B.K. (1997). *Prenatal and Neonatal Medicine,* **2**: 92-100.

Noy, Y. and Sklan, D. (1995). *Poultry Science,* **74**: 366- 373.

Packer, L. (1992). *Proceedings of the Society for Experimental Biology and Medicine,* **200:** 271-276.

Packer, L. and Landvik, S. (1989). In: *Vitamin E Biochemistry and Health Implications.* Eds. A.T. Diplock, L.J. Machlin, L. Packer and W.A. Pryor. New York Academy of Science, **570,** pp 1-6.

Rice-Evans, C.A., Samson, J., Bramley, P.M. and Holloway, D.E. (1997). *Free Radical Research,* **26**: 381-398.

Roch, C., Boulianne, M. and Roth, L. (2000). In: *Biotechnology in the Feed Industry.* Proceedings Alltech's 16th Annual Symposium. Eds T.P.Lyons and K.A. Jacques. Nottingham University Press, Nottingham, pp.261-276.

Schwarz, K.B. (1996). *Free Radical Biology and Medicine,* **21**: 641-649.

Sen, C.K. (2000). *Current Topics in Cell Regulation,* **36**: 1-30

Siddons, R.C. (1969). *Biochemical Journal*, **112**: 51-59.

Soto-Salanova, M.F. and Sell, J.L. (1995). *Poultry Science,* **74:** 201-204.

Soto-Salanova, M.F. and Sell, J.L. (1996). *Poultry Scienc,e* **75**,: 1393-1403.

Soto-Salanova, M.F., Sell, J.L., Mallarino, E.G., Piquer, F.J., Barker, D.L., Palo, P.E. and Ewan, R.C. (1993). *Poultry Science,* **72:** 1184-1188.

Surai, P.F.(1999). *Poultry and Avian Biology Reviews*, **10**: 1-60.

Surai, P.F. (1999a). *British Poultry Science,* **40**: 397-405.

Surai, P.F. (2000). *British Poultry Science,* **41**: 235-243.

Surai, P. and Ionov, I. (1994). *Journal fur Ornithologie*, **135:** 85.

Surai, P., Ionov, I. and Sakhatsky, N. (1993). In: *Proceedings of 10th International Symposium on Current Problems in Avian Genetics.* Nitra, Slovakia. p. 42.

Surai, P., Ionov, I., Kuchmistova, E., Noble, R.C. and Speake, B.K. (1998). *Journal of Science in Food and Agriculture,* **76**: 593-598.

Surai, P.F., Ionov, I.A., Kuklenko, T.V., Kostjuk, I.A., MacPherson, A., Speake, B.K., Noble, R.C. and Sparks, N.H.C. (1998a). *British Poultry Science,* **39:** 257-263.

Surai, P.F., McDevitt, R., Speake, B.K., Noble, R.C. and Sparks, N.H.C. (1999). *Proceedings of the Nutrition Society,* **58**: 29A.

Surai, P.F., Noble, R.C. and Speake, B.K. (1996). *Biochimica et Biochysica Acta,* **1304:** 1-10.

Surai, P.F., Noble, R.C. and Speake, B.K. (1999a). *British Poultry Science,* **40:** 406-410.

Surai, P.F. and Speake, B.K. (1998). *Journal of Nutritional Biochemistry,* **9**: 645-651.

Surai, P.F. and Speake, B.K. (1998a). *Comparative Biochemistry and Physiology,* **121B**: 393-396.

Surai, P.F., Speake, B.K., Noble, R.C. and Sparks, N.H.C. (1999b). *Biological Trace Element Research*, **68**: 63-78.

Turrens, J.F. (1997). *Bioscience Reports,* **17**: 3-8.

Vieira, S.L. and Moran, E.T. (1999). *World's Poultry Science Journal,* **55**: 125-142.

Wyatt, C.L., Weaver, W.D., Beane, W.L., Denbow, D.M. and Gross, W.B. (1986). *Poultry Science,* **65**: 2156-2164.