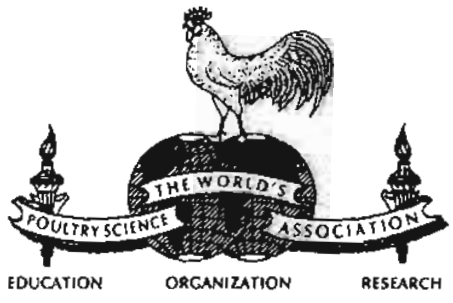
1ST Mediterranean Summit of WPSA

Advances and Challenges in Poultry Science

Book of Proceedings

Editors:
A. Tserveni-Goussi
A. Yannakopoulos
P. Fortomaris
G. Arsenos
E. Sossidou

07 – 10 MAY 2008
Porto Carras, Chalkidiki



Greek Branch

▼ MYCOTOXIN-ANTIOXIDANT INTERACTIONS: THEORETICAL CONSIDERATIONS AND PRACTICAL APPLICATIONS

[1-4]Peter F. Surai, [3]Miklos Mezes, [5]Julia E. Dvorska

[1] Scottish Agricultural College, Edinburgh, Scotland, UK;
[2] Univesity of Glasgow, UK;
[3] Szent István University, Gödöllő, Hungary and
[4] Trakia University, Stara Zagora, Bulgaria and
[5] Sumy National University, Ukraine, psurai@alltech.com

Keywords: mycotoxins, antioxidants, lipid peroxidation, apoptosis

ABSTRACT

A delicate balance between antioxidants and pro-oxidants in the body in general and specifically in the cell is responsible for regulation of various metabolic pathways leading to maintenance of immunocompetence, growth and development and protection against stress conditions associated with commercial poultry production. This balance can be regulated by dietary antioxidants, including vitamin E, carotenoids and selenium. On the other hand, nutritional stress factors have a negative impact on this antioxidant/pro-oxidant balance. In this respect mycotoxins are considered to be among most important feed-born stress factors. It is not clear at present if mycotoxins stimulate lipid peroxidation directly by enhancing free radical production or the increased tissue susceptibility to lipid peroxidation is a result of compromised antioxidant system. It seems likely that both processes are involved in this stimulation. In most cases lipid peroxidation in tissues caused by mycotoxins was associated with decreased concentrations of natural antioxidants.

It has been shown that T-2 toxin, aflatoxins, DON, fumonisins, zearalenon and other mycotoxins increased lipid peroxidation, cause apoptosis and are involved in gene expression regulation. In particular, genes involved in oxidative stress, apoptosis and immunity are affected by mycotoxins. Therefore practical approached to deal with mycotoxins include usage of effective absorbents and their combination with increased doses of natural antioxidants. In particular a combination of yeast wall derived mycotoxins binder Mycosorb with organic Se in the form of Sel-Plex is shown to be an effective means to deal with variety of mycotoxins.

INTRODUCTION

"Silent killers", "invisible thieves", unavoidable contaminants, natural toxicants - all these names have been given to a group of fungal metabolites called mycotoxins and their negative effects on animal production are incalculable. In general mycotoxins are considered to be unavoidable contaminants in foods and feeds and are a major problem all over the world. Interest in these naturally occurring chem-

ical compounds is intense due to their detrimental effect on human health (carcinogenicity) and animal productive and reproductive traits. More than 300 mycotoxins have been shown to induce signs of toxicity in mammalian and avian species and this number is increasing. It has been estimated that 25% of the world's crop production is contaminated with mycotoxins (Fink-Gremmels, 1999). The most significant mycotoxins in naturally-contaminated foods and feeds are aflatoxins, ochratoxins, zearalenone, T-2 toxin, vomitoxin and fumonisins. In many cases these mycotoxins can be found in combination in contaminated feed.

Mycotoxins appear at different stages of grain production. For example, Fusarium species are known to invade grains during the growth of the plant and they produce so-called "field mycotoxins". On the other hand, *Aspergillus* and *Penicillium* species generally develop during grain storage and so may be called "storage mycotoxins". This simple classification tends to over-simplify the situation. However, two facts are clear: mycotoxin contamination depends on moisture content of grain which should be less than 15% and drought stress can also increase fungal contamination of grain. In practice, a range of mycotoxins can be found in contaminated feeds, the type and level depending on climatic and storage conditions. Temperate climates with high moisture conditions, e.g. Canada, USA and Europe, encourage the growth of *Fusarium* and *Penicillium* species, and DON, zearalenone, ochratoxin A(OTA) and T-2 toxin that are of concern for human health. On the other hand, warm and humid climatic conditions, e.g. Latin America, Asian countries and some parts of Australia, are ideal for the growth of *Aspergillus* and the production of aflatoxin, considered to be a carcinogen. The winter season in these countries favours the production of zearalenone, DON, T-2 toxin, ochratoxin A, etc. Worldwide trade in feed ingredients leads to a wide distribution of the mycotoxins. Among all mycotoxins, those from *Fusarium* species are considered to be important contaminants of poultry feed (Table 1). Trichothecenes, zearalenone, fumonisins, moniliformin and fusaric acid are the major *Fusarium* mycotoxins occurring on a worldwide basis in cereal grains, animal feeds and forages. Furthermore, the trichothecene mycotoxins themselves comprise a vast group of over 100 fungal compounds with the same basic structure (Leeson *et al.*, 1995).

Acute mycotoxicosis outbreaks are rare events in modern poultry production, however, low mycotoxin doses which very often are not detected are responsible for reduced efficiency of production and increased susceptibility to infectious disease. Biochemical changes in mycotoxicosis vary greatly and lipid peroxidation and membrane disruption, apoptosis and compromised synthesis of DNA/protein are regarded as the most important consequences of mycotoxicosis (Surai, 2002; 2006).

INCREASED LIPID PEROXIDATION AS A CONSEQUENCE OF MYCOTOXICOSES

A delicate balance between antioxidants and pro-oxidants in the body in general and specifically in the cell is responsible for regulation of various metabolic pathways leading to maintenance of immunocompetence, growth and development and

Table 1. Effects of common mycotoxins (adapted from Surai, 2006).

Mycotoxin	Effects on poultry
Aflatoxin B1	- Decreased performance - Reduced egg production and hatchability - Decreased serum proteins - Increased liver and kidney weight - Liver and kidney lesions - Decreased semen volume and testes weights - Disruption of germinal epithelium - Decreased hatchability - Enlarged, fatty livers and enlarged spleens
Ochratoxin A	- Reduced weight gain and feed consumption - Impaired feed efficiency - Reduced egg production - Reduced egg quality - Induced immunosuppression - Kidney lesions
Fumonisin B1	- Rickets like deformities in chickens with leg weakness - Hepatocellular hyperplasia - Increased kidney and proventriculus weights - Liver lesions - Immunotoxicity
T-2 toxin, DON	- Reductions in feed consumption and weight gain - Severe oral lesions - Abnormal behaviour - Altered feathering - Decreased resistance to pathogens - Decreased egg production
Zearalenone	- Impaired shell quality - Decreased egg production

protection against stress conditions associated with commercial poultry production. This balance can be regulated by dietary antioxidants, including vitamin E, carotenoids and selenium. On the other hand, nutritional stress factors have a negative impact on this antioxidant/pro-oxidant balance. In this respect mycotoxins are considered to be among most important feed-born stress factors. It is not clear at present if mycotoxins stimulate lipid peroxidation directly by enhancing free radical production or the increased tissue susceptibility to lipid peroxidation is a result of compromised antioxidant system. It seems likely that both processes are involved in this stimulation. In most cases lipid peroxidation in tissues caused by mycotoxins was associated with decreased concentrations of natural antioxidants.

It has been shown that, OTA has a stimulating effect on lipid peroxidation. In most of cases, thiobarbituric acid reactive substances (TBARS) accumulation was used as a measurement of lipid peroxidation. Furthermore, ethane exhalation, EPR registered free radicals, hydroxyl radical formation, single-strand cleavage DNA, DNA adduct formation as well as LDH release were also used to confirm pro-oxidant properties of OTA. Various in vitro and in vivo systems were also used including liver microsomes, phospholipid vesicles, primary cell cultures, whole organs and whole body (Surai, 2006). T-2 toxin was also shown to have pro-oxidant properties. Those properties were confirmed with rat, mouse and quail liver tissue and yeast. TBARS accumulation was a method of choice for most of the studies, however, data on conjugate diene formation and DNA fragmentation also showed those effects. Effect of AFB1 on lipid peroxidation has been studied in rat liver and kidney as well as in cultured hepatocytes and in an in vitro model system. Similar to the examples above, TBARS accumulation was substantially increased as well as conjugate diene production. At the same time GSH concentration and activities of antioxidant enzymes significantly declined as a result of AFB1 action (for review see Surai and Dvorska, 2005). FB1 also stimulated lipid peroxidation in rat liver, rat liver nuclei fraction, primary rat hepatocytes, Vero cells in culture and PC bilayers. In those systems TBARS accumulation and DNA strand breaks were increased. DON increased TBARS formation in rat and mice liver and decreased GSH in rat brain and spleen. There are also data available indicating pro-oxidant properties of zearalenone and citrinin. Aurofusarin is shown to decrease antioxidant defences and stimulate lipid peroxidation in quail egg and tissues of newly hatched quail (Surai, 2002).

It is clear from the data that mycotoxins strongly promote lipid peroxidation in various *in vitro* and *in vivo* systems. This effect was obvious no matter which measurement was used to assess the process of lipid peroxidation.

MYCOTOXINS AND APOPTOSIS

The maintenance of tissue homeostasis involves the removal of superfluous and damaged cells. This process is often referred to as 'programmed cell death' or 'apoptosis' since it is thought that cells activate an intrinsic death program contributing to their own demise. Several processes, such as initiation of death signals at the plasma membrane, expression of pro-apoptotic oncoproteins, activation of death proteases, endonucleases etc., ultimately coalesce to a common irreversible execution phase leading to cell demise. A balance between cell death and cell survival factors plays a major role in the decision making process as to whether a cell should die or must live (for review see Surai, 2006).

Apoptosis is distinguishable from necrosis. When cell death is induced by osmotic, physical or chemical damage, early disruption of external and internal membranes takes place with subsequent liberation of denatured proteins into the cellular space and induction of an inflammatory response in the vicinity of the dying cell. In contrast, apoptosis is characterised by cell shrinkage, nuclear pyknosis, chro-

matin condensation, DNA cleavage into fragments of regular sizes and activation of the cystein-proteases called caspases. Reactive oxygen species are thought to play a major role in apoptosis, being involved in the initiation as well as execution of apoptosis. GSH depletion increases the percentage of apoptotic cells in a given population; and increased GSH concentration is shown to decrease the percentage of apoptosis in fibroblasts (Sastre *et al.*, 1996). In fact, GSH depletion sensitised cells for intracellular induction of apoptosis. Therefore, a decrease in GSH concentration, or an increase in GSSG concentration or perhaps a change in the ratio of GSH/GSSG constitutes a trigger for apoptosis. For example, ROS from mitochondria can cause apoptosis after GSH depletion. Therefore, apoptosis is induced by oxidative damage either directly from oxygen free radicals or hydrogen peroxide or from their generation in cells by injurious agents. For example, H_2O_2 is considered a common mediator for the apoptosis induced by various anticancer drugs (Simizu *et al.*, 1998). In line with those findings there are data showing protective effects of catalase and SOD from different inducers of apoptosis. Indeed, intracellular induction of apoptosis depends on ROS production and can be efficiently blocked by antioxidants (Langer *et al.*, 1996).

In many cases mycotoxins decreased cellular level of GSH, which can trigger apoptosis. In general, T-2 toxin is a most potent apoptotic agent. However, there are also reports indicating apoptosis caused by fumonisin B1 (FB1), OTA and aflatoxin B1 (AFB1; for review see Surai, 2002; 2006). For example, based on the DNA fragmentation profile in gel electrophoresis and the morphological changes in electron microscopy, the induction of apoptotic nuclear changes by various mycotoxins was investigated in HL-60 human promyelotic leukemia cells (Ueno *et al.*, 1995). The results showed that T-2 toxin, nivalenol, deoxynivalenol, OTA, citrinin, AFB1 and some other mycotoxins were positive for the induction of DNA fragmentation. However, fumonisin B1 was not active in DNA fragmentation. The toxicity of the mycotoxins nivalenol (NIV), DON and FB1 were studied in the K562 human erythroleukemia cell line (Minervini *et al.*, 2004). Morphological evidence of apoptosis was related to the toxicity of the mycotoxins and more toxic NIV and DON resulted in more late stage apoptotic events than FB1. The results suggested that DNA damage and apoptosis rather than plasma membrane damage and necrosis may be responsible for the observed cytotoxicity. Fumonisin B1, T-2 toxin, Fusarenon-X and Deoxynivalenol could induce apoptosis of liver cell, kidney cell, gastrointestinal epithelial cell, immunological cells as well as several cell lines of human and animals. Furthermore the rank order of the potency of trichothecene mycotoxins to induce internucleosomal DNA fragmentation was found to be T-2, satratoxin G, roridin A >> diacetoxyscirpenol > baccharin B-5 >> nivalenol, deoxynivalenol, 3-acetyldeoxynivalenol, fusarenon-X, baccharin B-4 vehicle control (Nagase *et al.*, 2001). The apoptosis-stimulating effect of FB1 has been clearly demonstrated and stimulation of lipid peroxidation by FB1 and decreased antioxidant concentrations including GSH in tissues could lead to changes in redox status of the cell and trigger a cascade of apoptotic changes.

In general, apoptosis is considered a common mechanism of toxicity of various mycotoxins. Since antioxidant-prooxidant balance in the cell (redox status) is responsible for regulation of apoptosis it seems likely that selenoproteins such as GSH-Px, TR and MSR could be potentially involved in prevention of mycotoxin-related apioptosis. Therefore, Se status of the animals could be an important factor in their resistance to mycotoxicoses.

MYCOTOXINS AND GENE EXPRESSION

Recently it has been suggested that toxic effects of various mycotoxins are mediated via changes in gene expression. For example, to examine morphological and gene expression changes induced by T-2 toxin in the fetal brain in detail, pregnant rats on day 13 of gestation were treated orally with a single dose of T-2 toxin (2 mg/kg) and sacrificed at 1, 3, 6, 9, 12 and 24 h after treatment (HAT). Microarray analysis showed that the expression of oxidative stress-related genes (heat shock protein 70 and heme oxygenase was strongly induced by T-2 toxin at 12 HAT, the peak time point of apoptosis induction (Sehata et al., 2004). The expression of mitogen-activated protein kinase (MAPK)-related genes (MEKK1 and c-jun) and other apoptosis-related genes (caspase-2 and insulin-like growth factor-binding protein-3 (IGF-BP3)) was also induced by the T-2 toxin treatment. Increased expression of oxidative stress- and apoptosis-related genes was detected in the liver of dams, placenta and fetal liver of pregnant rats treated with T-2 toxin at the peak time point of apoptosis (Sehata et al., 2005). Decreased expression of lipid metabolism- and drug-metabolizing enzyme-related genes was also detected in these tissues.

It was shown that OTA also can affect gene expression. Human renal cell cells were exposed to 50 μM OTA during 6 and 24 h, and gene expression profiles were analyzed (Arbillaga et al., 2007). In the experiment, few gene expression changes were identified at 6 h (179 genes), but many genes were differentially expressed at 24 h (2083 genes). Down-regulation was the predominant effect, with 90% and 67% of genes down-regulated at 6 and 24 h, respectively After 6 h, with slight cytotoxicity (83% survival), genes involved in mitochondrial electron transport chain were up-regulated; and after 24 h, with a more pronounced cytotoxicity (51% survival), genes implicated in oxidative stress response were also up-regulated. Increase in intracellular ROS level and oxidative DNA damage was evident at both exposure times being more pronounced with high cytotoxicity (Arbillaga et al., 2007). Exposure to OTA also significantly upragulated GSH-Px1 and GSH-Px3 as well as extracellular SOD, probably reflecting adaptive changes to stress. In another study using cDNA microarray technology, Luhe et al. (2003) showed that OTA induced changes on genes related to DNA damage response, apoptosis, inflammation and oxidative stress in rat kidney *in vivo* and in primary cultures of renal proximal tubular cells, *in vitro*. There is some evidence to suggest that oxidative stress in response to OTA may result from down-regulation of genes involved in antioxidant defence (Cavin et al., 2006 and Marin-Kuan et al., 2006). Indeed, many

affected genes are involved in chemical detoxication and antioxidant defense. The depletion of these genes is likely to impair the defense potential of the cells, resulting in chronic elevation of oxidative stress in the kidney. (Marin-Kuan et al., 2006).

It seems likely that DON and AFB1 are also involved in gene regulation changes. Mice were treated orally with 25 mg/kg body weight DON, and 2h later spleens were collected for macroarray analysis. Expression of 116 out of 1176 genes was significantly altered compared to average expression levels in all treatment groups. When genes were arranged into an ontology tree to facilitate comparison of expression profiles between treatment groups, DON was found primarily to modulate genes associated with immunity, inflammation, and chemotaxis (Kinser et al., 2004). The gene expression pattern of diploid yeast cells exposed to AFB(1) using high-density oligonucleotide arrays comprising specific probes for all 6218 open reading frames were analyzed (Keller-Seitz et al., 2004). Among 183 responsive genes, 46 are involved in either DNA repair or in control of cell growth and division. Eleven of the 15 inducible DNA repair genes, including RAD51, participate in recombination. Mice were fed control diets or diets containing 300 ppm FB1, Fusarium verticillioides culture material (CM) providing 300 ppm FB1. Hepatotoxicity found in FB1- and CM-fed mice and characterized by apoptosis, and cell proliferation. Transcript profiling using oligonucleotide arrays showed that CM and FB1 elicited similar expression patterns of genes involved in cell proliferation, signal transduction, and glutathione metabolism (Voss et al., 2006). Zearalenon is also involved in gene expression regulation (Yu et al., 2005) showing pro-prolliferative activity.

ARE THERE WAYS OF PREVENTING AGAINST DAMAGING AFFECTS OF MYCOTOXINS?

Since lipid peroxidation plays an important role in mycotoxin toxicity, a protective effect of antioxidants is expected. Indeed, in several experiments with various animal species, protective effects of antioxidants against toxic effects of mycotoxins were observed (for review see Surai, 2002). In spite of positive effects of natural antioxidants on animals fed mycotoxin contaminated diets, the most promising and practical approach has been the addition of adsorbents to contaminated feed. Mycotoxins can be bound to the adsorbent and pass harmlessly through the digestive tract. The extent to which various compounds bind specific toxins varies considerably. Many products only bind aflatoxin, leaving such mycotoxins as T-2 in the intestinal tract without alteration. A yeast cell wall-derived glucomannan (Mycosorb) has been shown to be effective against a wide range of mycotoxins. In our experiment it was shown that yeast glucomannans (Mycosorb) in T-2 toxin-containing diets fed quail significantly slowed the depletion of natural antioxidants and vitamin A in the liver (Dvorska and Surai, 2001). This protective effect can be attributed to the high adsorbent capability that esterified glucomannans have for T-2 toxin. It could well be that mycotoxin binding by Mycosorb also prevents T-

2 toxin participation in development of oxidative stress in the intestine. As a result, damage to the enterocytes is prevented thereby maintaining effective antioxidant absorption, assimilation and delivery to the target tissues. Protective effect of Mycosorb against oxidative stress in quail egg and embryo caused by consumption of aurofusarin was also shown (Dvorska et al., 2003). It seems likely that consumption of mycotoxins could compromise Se metabolism in animals. Therefore, a combined usage of adsorbents and antioxidants would show beneficial effects against mycotoxicosis. In fact, Se concentration in the quail liver was significantly reduced due to T-2 toxin consumption (Dvorska et al., 2007). Mycosorb inclusion into the diet showed a significant protective effect against Se depletion. However, a combination of Mycosorb and Sel-Plex was shown even more effective. Mycosorb also showed protective effects against vitamin E, carotenoids and reduced glutathione depletion in quail liver due to T-2 toxin consumption. Furthermore, lipid peroxidation in the liver of quail fed on T-2-supplemented diet was significantly reduced due to Mycosorb inclusion in the diet and a combination of Mycosorb and Sel-Plex was shown to give extra protection. However, despite positive effects on the birds, inclusion of Mycosorb in the quail or chicken diet was unable to completely prevent the adverse effects of T-2 toxin on the antioxidant systems of the liver of the growing quail; indicating that not all T-2 toxin was bound and released from the intestine. Therefore a combination of mycotoxin binders with natural antioxidants, in particular with Se and vitamin E, could be the next step in preventing damaging effects of mycotoxins on animals including poultry

CONCLUSIONS

Recent results show that in many cases membrane-active properties of various mycotoxins determine their toxicity. Indeed, incorporation of mycotoxins into membrane structures causes various detrimental changes. These changes are associated with alteration of fatty acid composition of the membrane structures and with peroxidation of long chain PUFAs inside membranes. This ultimately damages membrane receptors, causing alterations in second messenger systems; then to inactivation of a range of membrane-binding enzymes responsible for regulation of important pathways. Finally, this causes alterations in membrane permeability, flexibility and other important characteristics determining membrane function. Detrimental effects of mycotoxins on DNA, RNA and protein synthesis together with pro-apoptotic action further compromise important metabolic pathways. Consequently, changes in physiological functions including growth, development, reproduction etc. occur. An importance of lipid peroxidation in all these processes is confirmed by protective effects of natural antioxidants against mycotoxin toxicity. However, protective effects of antioxidants including selenium are of limited value and a combination of mycotoxin binders with organic selenium could be the next step in preventing damaging effects of mycotoxins in animal and poultry production.

REFERENCES

Arbillaga L, Azqueta A, van Delft JH, L_pez de Cerain A. (2007). In vitro gene expression data supporting a DNA non-reactive genotoxic mechanism for ochratoxin A. *Toxicol Appl Pharmacol.* **220**: 216-224.

Cavin C, Delatour T, Marin-Kuan M, Holzhäuser D, Higgins L, Bezençon C, Guignard G, Junod S, Richoz-Payot J, Gremaud E, Hayes JD, Nestler S, Mantle P, Schilter B. (2007). Reduction in antioxidant defenses may contribute to ochratoxin A toxicity and carcinogenicity. *Toxicol Sci.* **96**: 30-39.

Dvorska, J.E., Surai, P.F., Speake, B.K. and Sparks, N.H.C. (2003). Protective effect of Modified Glucomannans against aurofusarin-induced changes in quail egg and embryo. *Comparative Biochemistry and Physiology* **135**: 337-343.

Dvorska, J.E. and Surai, P.F. (2001). Effects of T-2 toxin, zeolite and Mycosorb on antioxidant systems of growing quail. *Asian-Australasian Journal of Animal Sciences* **14**: 1-6.

Dvorska JE, Pappas AC, Karadas F, Speake BK, Surai PF. (2007). Protective effect of modified glucomannans and organic selenium against antioxidant depletion in the chicken liver due to T-2 toxin-contaminated feed consumption. *Comparative Biochemistry and Physiology* **145C**: 582-587.

Fink-Gremmels, J. (1999). Mycotoxins: their implications for human and animal health. *Veterinary Quatery* **21**:115-120.

Keller-Seitz MU, Certa U, Sengstag C, Würgler FE, Sun M, Fasullo M. (2004). Transcriptional response of yeast to aflatoxin B1: recombinational repair involving RAD51 and RAD1. Mol Biol Cell. 15: 4321-4336.

Kinser S, Jia Q, Li M, Laughter A, Cornwell P, Corton JC, Pestka J. (2004). Gene expression profiling in spleens of deoxynivalenol-exposed mice: immediate early genes as primary targets. *J Toxicol Environ Health A.* **67**: 1423-1441.

Langer, C., Jurgensmeier, J.M. and Bauer, G. (1996). Reactive oxygen species act at both TGF-beta-dependent and -independent steps during induction of apoptosis of transformed cells by normal cells. *Experimental Cell Research* **222**: 117-124.

Leeson, S., Diaz, G., and Summers, J.D. (1995). *Poultry metabolic Disorders and Mycotoxins.* University Books. Guelph, Canada.

Marin-Kuan M, Nestler S, Verguet C, Bezençon C, Piguet D, Mansourian R, Holzwarth J, Grigorov M, Delatour T, Mantle P, Cavin C, Schilter B. (2006). A toxicogenomics approach to identify new plausible epigenetic mechanisms of ochratoxin a carcinogenicity in rat. *Toxicol Sci.* **89**:120-134.

Minervini F, Fornelli F, Flynn KM. (2004). Toxicity and apoptosis induced by the mycotoxins nivalenol, deoxynivalenol and fumonisin B1 in a human erythroleukemia cell line. *Toxicol In Vitro.* **18**: 21-28.

Nagase, M., Alam, M.M., Tsushima, A., Yoshizawa ,T. and Sakato, N. (2001). Apoptosis induction by T-2 toxin: activation of caspase-9, caspase-3, and DFF-40/CAD through cytosolic release of cytochrome c in HL-60 cells. *Bioscience, Biotechnology, and Biochemistry* **65**: 1741-1747.

Sastre, J., Pallardo, F.V. and Vina, J. (1996). Glutathione, oxidative stress and aging. *Age* **19**: 129-139

Sehata S, Kiyosawa N, Makino T, Atsumi F, Ito K, Yamoto T, Teranishi M, Baba Y, Uetsuka K, Nakayama H, Doi K. (2004). Morphological and microarray analysis of T-2 toxin-induced rat fetal brain lesion. *Food Chem Toxicol.* **42**: 1727-1736.

Sehata S, Kiyosawa N, Atsumi F, Ito K, Yamoto T, Teranishi M, Uetsuka K, Nakayama H, Doi K. (2005). Microarray analysis of T-2 toxin-induced liver, placenta and fetal liver lesions in pregnant rats. *Exp Toxicol Pathol.* **57**: 15-28.

Simizu, S., Takada, M., Umezawa, K. and Imoto, M. (1998). Requirement of caspase-3-like protease-mediated hydrogen peroxide production for apoptosis induced by various anticancer drugs. *Journal of Biological Chemistry* 273: 26900-26907.

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

Surai, P.F. (2002). Selenium in Nutrition and Health. Nottingham University Press, Nottingham, UK.

Surai, P.F. and Dvorska J.E. (2005). Effects of mycotoxins on antioxidant status and immunity. In: Diaz D. Ed: Mycotoxins. Nottingham University Press, Nottingham, pp. 93-137.

Ueno, Y., Umemori, K., Niimi, E., Tanuma, S., Nagata, S., Sugamata, M., Ihara, T., Sekijima, M., Kawai, K. and Ueno, I. (1995). Induction of apoptosis by T-2 toxin and other natural toxins in HL-60 human promyelotic leukemia cells. *Natural Toxins* 3: 129-137.

Voss KA, Liu J, Anderson SP, Dunn C, Miller JD, Owen JR, Riley RT, Bacon CW, Corton JC. (2006). Toxic effects of fumonisin in mouse liver are independent of the peroxisome proliferator-activated receptor alpha. *Toxicol Sci.* 89: 108-119.

Yu Z, Zhang L, Wu D, Liu F. (2005). Anti-apoptotic action of zearalenone in MCF-7 cells.*Ecotoxicol Environ Saf.* **62**: 441-446.