MYCOTOXINS AND ANIMAL HEALTH: FROM OXIDATIVE STRESS TO GENE EXPRESSION

MIKOTOKSINI I ZDRAVLJE ŽIVOTINJA: OD OKCIDACIJSKOG STRESA DO ISPOLJAVANJA GENA

P. F. Surai, M. Mezes, S. D. Melnichuk, Tatiana I. Fotina

Scientifiic review - Pregledno znanstveni članak
Received - Primljeno: 28. february - veljača 2008.

SUMMARY

Mycotoxin contamination of the feed and food is a global problem. There are several unresolved questions in this regard. Firstly, more than 25% of world grain production is contaminated by mycotoxins. In particular, Fusarium mycotoxins (so called field mycotoxins) contaminate up to 100% of the grain. Since these mycotoxins come from the field it is difficult to deal with them and various technological approaches including plant selection for mycotoxin resistance have not produced any significant results. Secondly, in nature there are more than 300 mycotoxins, but analytical techniques for routine mycotoxins analysis have been developed only for about 30 major mycotoxins. Therefore, if there is a conclusion from the analytical lab that "mycotoxins have not been found" this means that 10-30 mycotoxins analyzed were not found. As for others, there is no answer. Thirdly, sampling for mycotoxins analysis is extremely difficult and is an important source of errors. Fourthly, there are no safe levels of mycotoxins, because of synergistic interactions of many mycotoxins: several mycotoxins in low concentrations could cause more problems than a single mycotoxin at a higher dose.

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; 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 and reproduction occur. Importance of oxidative stress and 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 natural antioxidants could be the next step in preventing damaging effects of mycotoxins in animal and poultry production.

Key words: mycotoxins, animal health, oxidative stress, gene expression

Peter F. Surai, Scottish Agricultural College, Edinburgh, Scotland, UK; Univesity of Glasgow, UK; Szent István University, Gödöllő, Hungary and Trakia University, Stara Zagora, Bulgaria; Miklos Mezes, Szent István University, Gödöllő, Hungary; Sergey D. Melnichuk, National Agricultural University, Kiev, Ukraine; Tatiana I. Fotina, Sumy National Agrarian University, Ukraine.

MYCOTOXINS OF CONCERN

Three major economically important genera of mycotoxin-producing fungi are *Aspergillus, Penicillium* and *Fusarium*. It is necessary to take into account that there are many different species of each of the mentioned genera. For example, *Aspergillus* species comprise a group of more than 150 members. About 45 of them, 75 *Penicillium* species and 25 *Fusarium* species are known to produce mycotoxins. Furthermore, mycotoxins can be toxic at very low concentrations. 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, as well as DON, zearalenone, ochratoxin A and T-2 toxin that are of concern for animal and human health. On the other hand, warm and humid climatic conditions, e.g. in 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 development of zearalenone, DON, T-2 toxin, ochratoxin A, etc. Worldwide trade in feed ingredients leads to a wide distribution of the mycotoxins. The most significant mycotoxins in feeds are (Surai, 2006; Task Force Report, 2003):

Aflatoxins

- Highly oxygenated secondary metabolites produced by certain toxigenic strains of *Asprgillus flavus* and *Aspergillus parasiticus* growing on a variety of feedstuff, including maize, wheat, rice and cottonseed.
- There are four naturally occurring aflatoxins B1, B2, G1 and G2 and a range of their active metabolites with aflatoxin B1 (AFB1) being the most toxic and carcinogenic compound of this group of mycotoxins.
- They are unavoidable natural contaminants produced by specific moulds that invade a number of feedstuffs and basic foods.
- They affect a range of avian (chicken, turkey, duck, pheasant, quail) and mammalian (pigs, cattle, sheep, dog, cat, monkey and human) species as well as fish.
- This group of mycotoxins possesses high carcinogenic, teratogenic, mutagenic and immunosuppressive activities.

Ochratoxin A

- A secondary fungal metabolite produced by *Penicillium and Aspergillus* species of fungi during the storage of cereals, cereal products and other plant-derived products and as a result it is found in various compounded feeds, in cereal grains (wheat, barley, corn, oats) and dry beans.
- It affects pig, chicken, duck, dog, rat and human.
- It is immunosuppressive, genotoxic, teratogenic and carcinogenic to monogastric animals. The most prominent effect of OA being nephrotoxicity.
- Feed contamination with OA typically results in increased mortality, poor feed conversion, poor growth rates and feed refusal. Post mortem examination revealed various detrimental changes including renal carcinogenity and hepatotoxicity .

T-2 toxin

- The trichothecene group of mycotoxins accounts for over one hundred fungal metabolites, of which T-2 toxin, produced by the *Fusarium* fungus, was the first to be studied.
- It contaminates corn, wheat, barley and oats.
- It affects a range of animal species including swine, cattle, chicken, turkey, horse, dog, cat, mouse, rat and human.

- The adverse effects of trichothecene toxins on animal health is expressed in a diverse range of symptoms including skin lesions, immunosuppression, hepatotoxicity, nephrotoxicity, neurotoxicity, genotoxicity and even death.
- The damage caused by trichothecene mycotoxins results primarily from the toxin's interruption of cell division in bone marrow, immunocompetent organs and intestinal mucosa, resulting in a serious immunosuppressive effect .
- T-2 toxin also has a strong inhibitory effect on protein synthesis, which in turn results in the inhibition of DNA and RNA synthesis.

Vomitoxin (Deoxynivalenol, DON)

- DON is produced world-wide by the *Fusarium* genus (*Fusarium graminearum and Fusarium culmorum*).
- It contaminates different cereal crops (wheat, maize and barley) used for food and feed production.
- It is one of the least acutely toxic trichothecenes.
- The main toxic effect is associated with inhibition of protein synthesis via binding to the ribosome.
- DON in moderate to low doses can cause a number of detrimental effects associated with reduced performance and immune function.
- The main effect at low dietary doses is a reduction in food consumption (anorexia), while higher doses induce vomiting (emesis).
- It alters brain neurochemicals.
- Animals fed low to moderate doses are able to recover from initial weight losses, while higher doses induce more long-term changes in feeding behaviour.
- Swine are more sensitive to DON than mice, poultry, and ruminants, with males being more sensitive than females.

Fumonisin B1

- The fumonisins are the most recently discovered family of aminopolycarboxylic acids produced predominantly by *Fusarium moniliforme,* but also by *F. proliferatum and F. napiforme*.
- Fumonisins are detected mainly in maize and maize-based diets.
- Several different derivatives of this mycotoxin have been described of which fumonisin B1 (FB1) is recognised as the most toxic.
- Toxic effects of FB1 are associated with the fact that it resembles the structure of cellular sphingolipids and therefore impairs ceramide synthesis by inhibiting ceramide synthetase.
- FB1 is highly toxic and has been shown to be responsible for some major toxicological effects in animals, including pigs and equine species but has comparatively low toxicity for poultry. In particular, FB1 causes porcine pulmonary oedema with severe lung oedema and hydrothorax and equine leukoencephalomalacia.

Zearalenone (Zea)

- It contaminates corn, hay and pelleted commercial feed.
- It affects swine, dairy cattle, chicken, turkey, lamb, rat, mouse and guinea pigs.
- Zea causes a variety of toxic effects in both experimental animals and livestock, and possibly, in humans.
- It is a stable compound, both during storage/milling and the processing/cooking of food.
- It is fairly rapidly absorbed following oral administration.
- It competitively binds to estrogen receptors in a number of *in vitro* systems and in the uterus, mammary gland, liver and hypothalamus of different species.
- Zea alters various immunological parameters.
- It causes alterations in the reproductive tract of laboratory animals and domestic animals causing decreased fertility, increased embryolethal resorptions, reduced litter size, changed weight of adrenal, thyroid and pituitary glands and change in serum levels of progesterone and estradiol.
- Teratogenic effects of Zea were found in pigs and sheep.

Molecular mechanisms of mycotoxin action include four major points (Surai, 2006):

- Inhibition of protein, RNA and DNA synthesis and DNA adduct formation;
- Membrane structure alteration, induction of oxidative stress and lipid peroxidation;
- Induction of programmed cell death (apoptosis);
- Gene expression changes.

OXIDATIVE STRESS 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 protection against stress conditions associated with commercial animal/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 is associated with decreased concentrations of natural antioxidants.

It has been shown that, OTA, T-2 toxin, DON, aflatoxins, fumonisins and zearalenon impose an oxidative stress and have a stimulating effect on lipid peroxidation. In most 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 mycotoxins. Various in vitro and in vivo systems were also used including liver microsomes, phospholipid vesicles, primary cell cultures, whole organs and whole body. TBARS accumulation was substantially increased and at the same time vitamin E and GSH concentrations and activities of antioxidant enzymes significantly declined as a result of mycotoxicosis.

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 characterized by cell shrinkage, nuclear pyknosis, chromatin 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 sensitises cells for intracellular induction of apoptosis.

In many cases mycotoxins decrease 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 DON, FB1, OTA and AFB1 (for review see Surai, 2002; 2006). Furthermore the rank order

of the potency of trichothecene mycotoxins to induce internucleosomal DNA fragmentation was found to be T-2 toxin, satratoxin G, roridin A >> diacetoxyscirpenol > baccharin B-5 >> nivalenol, deoxynivalenol, 3-acetyldeoxynivalenol, fusarenon-X, baccharin B-4 vehicle control (Nagase *et al*., 2001).

In general, apoptosis is considered as a common mechanism of toxicity of various mycotoxins. Since antioxidant-prooxidant balance in the cell (redox status) is responsible for a regulation of apoptosis it seems likely that natural antioxidants and selenoproteins such as GSH-Px, thioredoxin reductase and methionine sulfoxide reductase B could be potentially involved in prevention of mycotoxin-related apoptosis. 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 and it seems likely that it is a characteristic of major mycotoxins.

T-2 Toxin

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) were also induced by the T-2 toxin treatment. From the results of microarray analysis and histopathological examination, T-2 toxin seems to induce oxidative stress in these tissues, following the changes in metabolism-related genes expression. These changes may alter the intracellular environments resulting in the induction of apoptosis (Sehata et al., 2004a). Furthermore, Sehata et al. (2005) suggested that the mechanism of T-2 toxin-induced toxicity in rats was due to oxidative stress followed by the activation of the MAPK pathway, finally inducing apoptosis. They showed increased expression of oxidative stress- and apoptosis-related genes in the liver of dams, placenta and fetal liver of pregnant rats treated with T-2 toxin at the peak time point of apoptosis. Decreased expression of lipid meta-bolism- and drug-metabolizing enzyme-related ge-nes was also detected in these tissues. In addition, increased expression of the c-jun gene was consistently observed in these tissues and it could well be that the c-jun gene may play an important role in T-2 toxin-induced apoptosis.

In an experiment conducted in the Institute of Endemic Disease in China it was shown that T-2 toxin induced a time-dependent and dose-dependent inhibition of cellular proliferation in human chondrocytes. Evidence of various steps of apoptotic cell death and shrinkage of chondrocytes, as well as margination and condensation of nuclear chromatin, were found based on electron microscopic observations (Chen eta al., 2006). It was shown that the apoptosis induced by T-2 toxin involved an increased Bax/Bcl-2 ratio. In fact, Bcl-2 mRNA expression remained unchanged in chondrocyte apoptosis induced by T-2 toxin treatment, while Bax mRNA expression increased following treatment with T-2 toxin. Indeed Bcl-2 is a member of a family of genes that regulates apoptosis. The family includes members, responsible for death suppression by production of anti-apoptotic proteins (Bcl-2 and Bcl-xl), and death promotion by producing pro-apoptotic proteins (Bax, Bak, Bik and Bcl-Xs). It is believed that the ratio of Bax to Bcl-2 protein can determine whether cells will die via apoptosis or be protected from it. In particular, Bcl-2 homodimers can prevent apoptosis by prevention of activation of the caspase cascade, blocking the loss of mitochondrial membrane potential, inhibiting release of cytochrome *c* from mitochondria. High levels of Bax relative to Bcl-2 after a death signal may increase the cell's susceptibility to apoptosis (Chen et al., 2006). The expression of apoptosis-related genes (c-*fos* and c-*jun*) mRNAs markedly increased before the development of apoptosis in the T-2 toxin-treated keratinocytes primary cultures (Albarenque and Doi, 2005). It is necessary to underline that it is believed that c-*fos* belongs to immediate-early response genes, and its activation with other factors such as c-

jun is considered to be an early response to cell injury, resulting in an increased sensitivity of keratinocytes to apoptosis. In the same study it was also shown that the expression of pro-inflammatory cytokines TNF-α mRNA and IL-1β mRNA increased in the T-2 toxin-treated keratinocytes cultures throughout the experimental period. Similar increases in the expression of TNF-α and IL-1β mRNA were also observed after T-2 toxin application in earlier in vivo experiments (Albarenque et al., 2001).

DON

The effects of the ribotoxic trichothecene deoxynivalenol (DON) on mitogen-activated protein kinase (MAPK)-mediated IL-8 expression were investigated in cloned human monocytes and peripheral blood mononuclear cells (PBMC; Islam et al., 2006). It was shown that DON (250 to 1000 ng/ml) induced both IL-8 mRNA and IL-8 heteronuclear RNA (hnRNA), an indicator of IL-8 transcription, in the human U937 monocytic cell line in a concentration-dependent manner. Expression of IL-8 hnRNA, mRNA and protein correlated with p38 phosphorylation and was completely abrogated by the p38 MAPK inhibitor SB203580. Zhou et al. (2005) reported that DON can induce phospho-p53 and p21 protein expression at 30 min after 250 ng/ml in macrophage cells, but the epithelium did not respond to DON by inducing p53 and p21 protein in that early time point. Furthermore, DON was proven to arrest G_2/M phase in the human intestinal epithelial cells via elevated p21 gene expression (Yang et al., 2008). Signaling pathways associated with DON-induced p21 gene expression included PI3 kinase and ERK1/2 MAP kinase cascade. It should be noted that up-regulation of p21 gene expression is generally associated with G1 phase arrest by DNA damage or cellular senescence, which is under the transcriptional control of p53 protein. DON-induced chemokine interleukin (IL)-8 expression is likely to be mediated at the transcriptional level by NF-kappaB, specifically p65, but does not appear to involve mRNA stabilization (Gray and Pestka, 2007). Indeed, when NF-kappaB subunit binding to a specific IL-8 promoter probe was evaluated by enzyme-linked immunosorbent assay DON was observed to increase p65 binding by 21-fold. Similarly DON induced pro-inflammatory IL-1beta, IL-6, and TNF-alpha gene expression (Pestka and Zhou, 2006). It is well accepted that TNF-α and IL-6 mediate injurious inflammatory processes and IL-1 is associated with leukocyte apoptosis (Islam and Pestka, 2003). 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).

OTA

It was shown that OTA could also affect gene expression. Human renal 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 defence. The depletion of these genes is likely to impair the defence potential of the cells, resulting in chronic elevation of oxidative stress in the kidney (Marin-Kuan et al., 2006).

Other mycotoxins

It seems likely that AFB1 is also involved in gene regulation changes. 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. There is also evidence that FB1 is also able to change gene expression. For example, mice were fed control diets or diets containing 300 ppm FB1 or Fusarium verticillioides culture material (CM) providing 300 ppm FB1. Hepatotoxicity found in FB1- and CM-fed mice 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.

CONCLUSIONS

The wide range of mycotoxins that can contaminate animal/poultry feed and their different chemical compositions make protection against mycotoxin-related toxicity a difficult task. There are several problems that complicate mycotoxin prevention issues:

- In many cases, the low levels of mycotoxins remain undetected in feed ingredients and their effects may also go unseen. For example, a decrease in hatchability by 0.5% would be difficult to notice. Detrimental effects on the immune system would be even more difficult to assess. However, the immunosuppressive activities of the mycotoxins have become of particular concern in EU countries after a ban on feed-grade antibiotics.
- Very often, a combination of various mycotoxins are present in the feed because the various fungal species can produce several toxins. A combination of several mycotoxins in low doses can have a bigger detrimental effect than a single mycotoxin at a higher dose.
- Mycotoxins can contaminate practically all feed ingredients. For example, Fusarium species have been found in wheat, maize, barley, oats and rye. On the other hand, aflatoxins can also contaminate oilseeds and other feed ingredients.
- There are no safe doses of mycotoxins. A dose that does not affect animal at short exposure could be toxic at longer consumption. Doses that may be safe under laboratory conditions can have detrimental effects on growth and reproduction under conditions of commercial poultry production.
- International trade of feed ingredients, e.g. maize and soybeans, especially long shipments from Latin America to European and Asian countries, is another important risk factor.
- Most mycotoxins are stable compounds that do not degrade during storage, milling or high-temperature feed manufacturing processes.

REFERENCES

1. Albarenque, S. M., Suzuki, K., Nakayama, H., Doi, K. (2001): Kinetics of cytokines mRNAs expression in the dorsal skin of hypotrichotic WBN/ILA-Ht rats following topical application of T-2 toxin, *Exp. Toxicol. Pathol.* 53: 271–274.
2. Albarenque, S. M., Doi, K. (2005): T-2 toxin-induced apoptosis in rat keratinocyte primary cultures. *Experimental and Molecular Pathology 78*: 144-149.
3. Arbillaga, L., Azqueta. A., van Delft, J. H., López 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.
4. Cavin, C., Delatour, T., Marin-Kuan, M., Holzhäuser, D., Higgins, L., Bezençon, C., Guignard, G., Junod, S., Richoz-Payot, J., Gremaud, E., Hayes, J. D., Nestler, S., Mantle, P., Schilter B. (2007): Reduction in antioxidant defenses may contribute to ochratoxin A toxicity and carcinogenicity. *Toxicol Sci.* 96: 30-39.
5. Chen, J., Chu, Y., Cao, J., Yang, Z., Guo, X., Wang, Z. (2006): T-2 toxin induces apoptosis, and selenium partly blocks, T-2 toxin induced apoptosis in chondrocytes through modulation of the Bax/Bcl-2 ratio. *Food Chem Toxicol.* 44: 567-573.
6. Gray, J. S., Pestka, J. J. (2007): Transcriptional regulation of deoxynivalenol-induced IL-8 expression in human monocytes. *Toxicol Sci*. 99: 502-511.

7. Islam, Z., Gray, J. S. and Pestka, J. J. (2006): p38 Mitogen-activated protein kinase mediates IL-8 induction by the ribotoxin deoxynivalenol in human monocytes. *Toxicol Appl Pharmacol*. 213: 235-244.
8. Islam, Z., Pestka, J. J. (2003): Role of IL-1(beta) in endotoxin potentiation of deoxynivalenol-induced corticosterone response and leukocyte apoptosis in mice. *Toxicol. Sci.* 74: 93–102.
9. Keller-Seitz, M. U., Certa, U., Sengstag, C., Würgler, F. E., Sun, M., Fasullo, M. (2004): Transcriptional response of yeast to aflatoxin B1: recombinational repair involving RAD51 and RAD1. *Mol Biol Cell.* 15: 4321-4336.
10. Kinser, S., Jia, Q., Li, M., Laughter, A., Cornwell, P., Corton, J. C., 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.
11. Luhe, A., Hildebrand, H., Bach, U., Dingermann, T., Ahr, H. J. (2003): A new approach to studying ochratoxin A (OTA)-induced nephrotoxicity: expression profiling in vivo and in vitro employing cDNA microarrays. *Toxicol Sci*. 73: 315-328.
12. 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.
13. Nagase, M., Alam, M. M., Tsushima, A., Yoshizawa ,T., 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.
14. Pestka, J., Zhou, H. R. (2006): Toll-like receptor priming sensitizes macrophages to proinflammatory cytokine gene induction by deoxynivalenol and other toxicants. *Toxicol Sci*. 92: 445-455.
15. Sastre, J., Pallardo, F. V., Vina, J. (1996): Glutathione, oxidative stress and aging. *Age* 19: 129-139.
16. 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.
17. 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.
18. Sehata, S., Kiyosawa, N., Sakuma, K., Ito, K., Yamoto, T., Teranishi, M., Uetsuka, K., Nakayama, H., Doi, K. (2004a): Gene expression profiles in pregnant rats treated with T-2 toxin. *Exp Toxicol Pathol.* 55: 357-366.
19. Surai, P. F. (2002): Natural Antioxidants in Avian Nutrition and Reproduction. Nottingham University Press, Nottingham, UK .
20. Surai, P.F. (2006): Selenium in Nutrition and Health. Nottingham University Press, Nottingham, UK.
21. Voss, K. A., Liu, J., Anderson, S. P., Dunn, C., Miller, J. D., Owen, J. R., Riley, R. T., Bacon, C. W., Corton, J. C. (2006): Toxic effects of fumonisin in mouse liver are independent of the peroxisome proliferator-activated receptor alpha. *Toxicol Sci.* 89: 108-119.
22. Yang, H., Chung, D. H., Kim, Y. B., Choi, Y. H., Moon, Y. (2008): Ribotoxic mycotoxin deoxynivalenol induces G2/M cell cycle arrest via p21Cip/WAF1 mRNA stabilization in human epithelial cells. *Toxicology.* 243: 145-154.
23. Yu, Z., Zhang, L., Wu, D., Liu, F. (2005): Anti-apoptotic action of zearalenone in MCF-7 cells.*Ecotoxicol Environ Saf.* 62: 441-446.
24. Zhou, H. R., Islam, Z., Pestka, J. J. (2005): Induction of competing apoptotic and survival signaling pathways in the macrophage by the ribotoxic trichothecene deoxynivalenol, *Toxicol. Sci.* 87: 113-122.

SAŽETAK

Kontaminiranje hrane mikotoksinima je globalni problem. S tim u svezi nekoliko je neriješenih pitanja. Prvo, više od 25% svjetske proizvodnje žitarica kontaminirano je mikotoksinima. Osobito mikotoksini fuzarijuma (tzv. poljski mikotoksini) kontaminiraju do 100% žitarica. Budući da ovi mikotoksini dolaze iz polja teško ih je suzbiti pa i različiti tehnički postupci uključujući i selekciju biljaka na otpornost na mikotoksine nisu dali značajnije rezultate. Drugo, razvijene analize odnose se samo na oko 30 važnijih mikotoksina. Zato ako je i nalaz analitičkog laboratorija da "nisu nađeni mikotoksini" znači da nije nađeno 10-30 analiziranih mikotoksina. Što se tiče ostalih, nema

odgovora. Treće, uzorkovanje za analizu mikotoksina vrlo je teško i važan je izvor pogrešaka. Četvrto, nema sigurnih razina mikotoksina zbog sinergetskih interakcija mnogih mikotoksina: nekoliko mikotoksina u malim koncentracijama može prouzročiti više problema nego jedan štetan u većoj količini.

Noviji rezultati pokazuju da u mnogo slučajeva svojstva aktivne membrane raznih mikotoksina određuju njihovu toksičnost. Doista, ugrađivanje mikotoksina u strukture membrane uzrokuje razne štetne promjene. Te su promjene povezane s promjenom sastava masnih kiselina strukture membrane i s peroksidacijom drugog lanca PUFA-e u membrani. Ovo konačno oštećuje receptore membrane, prouzročivši promjene drugog niza enzima koji povezuju membranu i odgovorni su za reguliranje važnih puteva. Konačno, to prouzrokuje promjene u propusnosti membrane, fleksibilnosti i drugim važnim značajkama koje određuju funkciju membrane. Štetno djelovanje mikotoksina na DNK, RNA i sintezu bjelančevina zajedno s proapoptotičnim djelovanjem dalje djeluje na važne metaboličke puteve. Konačno dolazi do promjene u fiziološkim funkcijama, uključujući rast, razvoj i reprodukciju. Važnost oksidacijskog stresa i peroksidacije lipida u svim tim procesima potvrđuje zaštitno djelovanje prirodnih antioksidanata protiv toksičnosti mikotoksina. Međutim, zaštitno djelovanje antioksidanata, uključujući selen, ograničene je vrijednosti i kombinacija. Vezanje mikotoksina s prirodnim antioksidantima mogao bi biti sljedeći korak u sprječavanju štetnog djelovanja u proizvodnji životinja i peradi.

Ključne riječi: mikotoksini, zdravlje životinja, oksidacijski stres, ekspresija gena