Impact of mycotoxins on the body's antioxidant defence

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Introduction

Mycotoxins are unavoidable contaminants in foods and feeds and are a major problem all over the world (Wood, 1992). The number of mycotoxins known to induce signs of toxicity in mammalian and avian species exceeds 300 (Fink-Gremmels, 1999; Leeson *et al.*, 1995) and is steadily increasing. The most significant mycotoxins in naturally-contaminated foods and feeds are aflatoxins, ochratoxins, zearalenone, T-2 toxin, vomitoxin and fumonisins (Devegowda *et al.*, 1998) and in many cases these mycotoxins can be found in combination in contaminated feed.

Among all mycotoxins, those from *Fusarium* species are considered to be important contaminants of poultry feed. Trichothecenes, zearalenone, fumonisins, moniliformin and fusaric acid are the major *Fusarium* mycotoxins occurring on a worldwide basis in cereal grains, animal feeds and forages (D'Mello *et al.*, 1999; Figure 1). 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 levels of mycotoxin contamination, which very often are not detected, are responsible for reduced efficiency of production and increased susceptibility to infectious disease. These sometimes undetectable or unseen compounds can cost poultry producers a fortune, unless a radical solution for the problem is found. The problem is further complicated since in many cases molecular mechanisms of their action have not been fully elucidated.

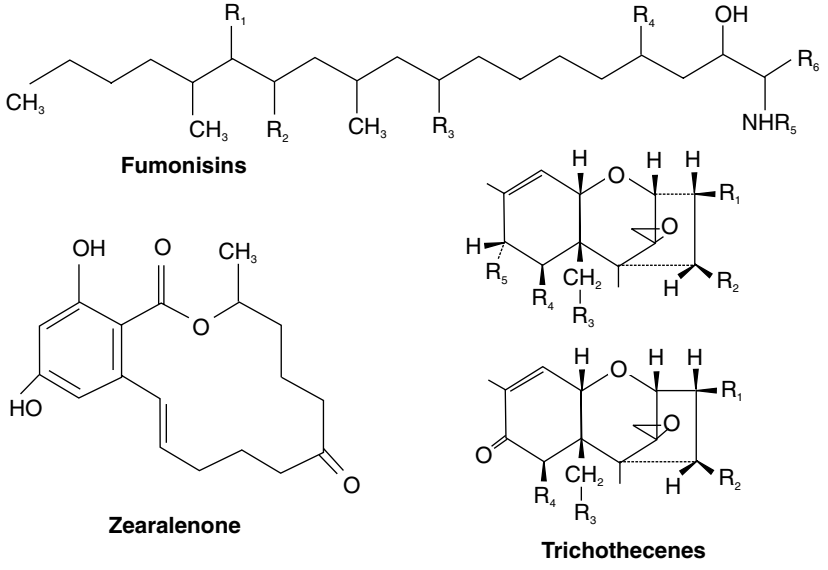

Figure 1. Diverse structure of some common mycotoxins.

Biochemical changes in mycotoxicosis vary greatly and lipid peroxidation is regarded as one of the most important consequences of mycotoxicosis (Mezes *et al.*, 1999). Aflatoxin B1 (AFB1), T-2 toxin, ochratoxin A (OA), fumonisin B1 (FB1), zearalenone (ZEN) and deoxynivalenol (DON) are the most notorious and extensively studied mycotoxins (Galvano *et al.*, 2001) and all of them are involved in lipid peroxidation.

Compromised antioxidant defences due to 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 poultry production (Surai and Dvorska, 2001). This balance can be regulated by dietary antioxidants, including vitamin E (Surai *et al.*, 1999), carotenoids (Surai and Speake, 1998; Surai *et al.*, 2001) and selenium (Se) (Surai, 2000). On the other hand, nutritional stress factors have a negative impact on this antioxidant/pro-oxidant balance. In this respect, mycotoxins can be considered among the most important feed-borne stress factors.

It is not clear at present whether mycotoxins stimulate lipid peroxidation directly by enhancing free radical production or if the increased tissue susceptibility to lipid peroxidation is a result of compromising antioxidant defence. It seems likely that both processes are involved. In most cases lipid peroxidation in tissues caused by mycotoxins was associated with decreased concentrations of natural antioxidants. For example, in an experiment with quail, levels of the primary liver antioxidants (α-tocopherol, γ-tocopherol, carotenoids and ascorbic acid) significantly decreased as a result of T-2 toxin consumption (Dvorska and Surai, 2001; Figure 2). Similarly, the presence of T-2 toxin in the diet decreased liver concentration of α-tocopherol in chickens (Hoehler and Marquardt, 1996). T-2 toxin consistently depressed concentrations of vitamin E in chicken plasma (Coffin and Combs, 1981).

Similarly, AFB1 in the feed interfered with the accumulation of carotenoids in chicken tissues (Schaeffer *et al.*, 1988), inducing pale bird syndrome. In fact, AFB1 caused a significant depression of lutein in the toe web, liver, serum and mucosa (Schaeffer *et al.*, 1988). Pigment restoration was accomplished by feeding the same diet supplemented with lutein (70 mg/kg). In young chickens AFB1 reduced the lutein content of the jejunal mucosa up to 35% and serum lutein was reduced up to 70% (Tyczkowski and Hamilton, 1987a), suggesting that AFB1 interfered with the absorbtion, transport and deposition of carotenoids. More precisely, AFB1 impaired lutein absorption in chickens (Tyczkowski and Hamilton, 1987b). In similar fashion, OA was shown to affect carotenoid assimilation in chickens. Again, depression in uptake

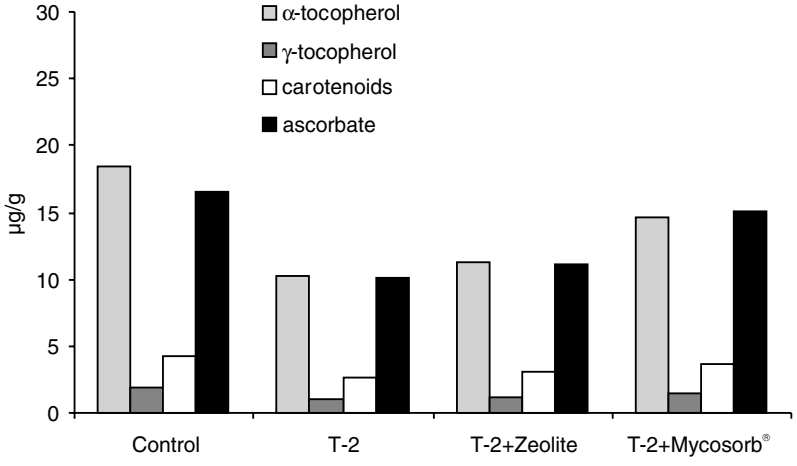

Figure 2. Effect of T-2 toxin and absorbents on antioxidants concentrations in quail liver (from Dvorska and Surai, 2001).

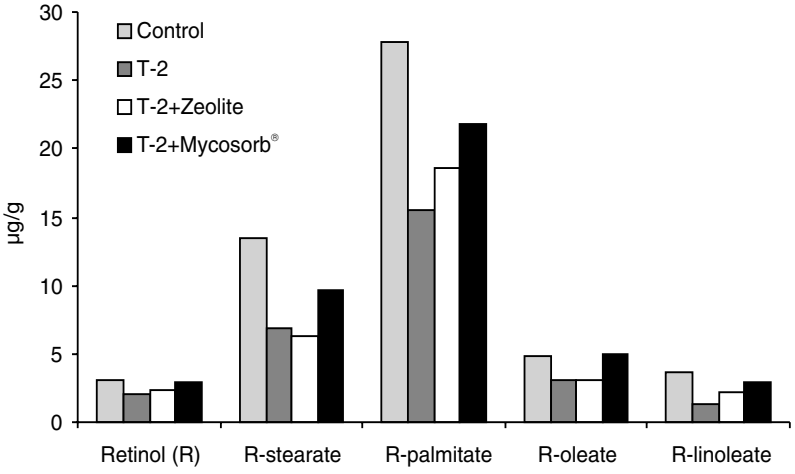

Figure 3. Effect of T-2 toxin and sorbents on vitamin A (retinol and retinol esters) concentrations in quail liver (from Dvorska and Surai, 2001).

of carotenoids by intestinal mucosa and depressed transport in serum were considered important mechanisms of AFB1 action on carotenoid metabolism (Schaeffer *et al.*, 1987; Huff and Hamilton, 1975).

In general, malabsorption syndrome is considered a common result of mycotoxicoses. For example, aflatoxicosis, ochratoxicosis and T-2 toxicosis were produced by feeding diets containing graded concentrations of the appropriate toxin to broiler chickens from hatching until 3 weeks of age (Osborne *et al.*, 1982). In this experiment AFB1, levels lower than those needed for growth inhibition produced a malabsorption syndrome characterized by steatorrhea, hypocarotenoidemia, and decreased concentrations of bile salts and pancreatic enzymes. T-2 toxin also produced malabsorption, but at concentrations higher than required to inhibit growth. Ochratoxicosis produced mainly hypocarotenoidemia (Osborne *et al.*, 1982). It is postulated that the decreased level of vitamin A in the quail liver as a result of T-2 toxin consumption (Dvorska and Surai, 2001; Figure 3) is also a reflection of the decreased intestinal absorption of fat soluble nutrients. Molecular mechanisms of malabsorption development due to mycotoxins need further investigation. However, it can be suggested that mycotoxins may stimulate lipid peroxidation in enterocytes leading to damage that could substantially contribute to malabsorption development.

The presence of ochratoxin A in the diet significantly decreased the concentration of α-tocopherol in the chicken liver (Hoehler and Marquardt, 1996). Furthermore, aflatoxin-treated barrows had decreased serum tocopherol and retinol concentrations compared with control and pre-test values, and decreased tocopherol concentration in cardiac tissue (Harvey *et al.*, 1994).

A pro-oxidant effect of mycotoxins in many cases could be mediated via changes in reduced glutathione concentration. For example, Rizzo *et al.* (1994) demonstrated that T-2 toxin decreased GSH content in rat liver. Treatment of fasted mice with a single dose of T-2 toxin (1.8 or 2.8 mg/kg body weight) by oral gavage led to marked decrease in hepatic GSH levels (Atroshi *et al.*, 1997). In male broiler chicks, hepatic GSH concentration decreased after 7 days of treatment (1.5 mg T-2 toxin/kg body weight/day) (Leal *et al.,* 1999). Acute exposure of mice to T-2 toxin (4 mg/kg,) resulted in a progressive decrease in hepatic glutathione content, reaching a minimum 6-8 hrs after toxin administration (Fricke and Jorge, 1991). Intraperitoneal administration of AFB1 to rats (2 mg/kg) was also associated with decreased GSH in the liver. In contrast, in 3-week-old male chickens daily aflatoxin gavage (2 mg/kg body wt, in corn oil) for 5 and 10 days elevated plasma hepatic GSH; and renal GSH was elevated after 10 days of aflatoxin treatment (Beers *et al.*, 1992a). Similarly, hepatic GSH increased 2 and 8 hrs following a single AFB1 dose and continued to increase through five daily doses of AFB1 (Beers *et al.*, 1992b).

There was GSH depletion in cultured rat hepatocytes as a result of AFB1 toxicosis (Liu *et*

al., 1999). Similarly, consumption of OA for two weeks was associated with a depletion of GSH from the mouse liver (Atroshi *et al.*, 2000). The mycotoxin patulin also decreased GSH concentration in rat hepatocytes (Busbee *et al.*, 1999). Since glutathione is responsible for the maintenance of cellular redox status (Sies, 1999) and therefore participates in regulation of gene expression (Arrigo, 1999), changes in GSH status could be detrimental.

One of the most important mycotoxin actions is their effect on antioxidant enzymes. Depending on experimental conditions (species, dose, route and duration of exposure, concentrations of other antioxidants etc.), antioxidant enzyme activities can increase in response to oxidative stress or decrease by direct or indirect action of mycotoxins. For example, treatment of pig kidney cells with 50 μM FB1 for 24 hrs significantly decreased cellular GSH and increased the activity of glutathione reductase (Kang and Alexander, 1996). The activities of GSH-Px, catalase and Cu/Zn-SOD were not changed by this treatment. Oral administration of T-2 toxin to rats (1.25 mg/kg) for five days decreased activity of liver glutathione-S-transferase (Ahmed and Ram, 1986). In contrast, feeding a single dose of T-2 toxin (2 mg/kg body weight) to rats was associated with increased activities of GSH-shuttle enzymes including GSH-Px, glutathione reductase and glucose-6-phosphate dehydrogenase (Suneja *et al.*, 1989), probably reflecting an adaptive response to oxidative stress. On the other hand, when male rats were fed a diet deficient in vitamins C and E and Se were orally administered a single dose of DON or T-2 toxin, there was a significant decrease in activities of GSH-Px, catalase, SOD and glutathione reductase (Rizzo *et al.*, 1994). Activity of GSH-Px in rat blood was decreased due to consumption of AFB1 (Choi *et al.*, 1995). Administration of AFB1 to rats (2 mg/kg intraperitoneally) decreased the activities of SOD, catalase, GSH-Px, glutathione-S-transferase and glutathione reductase in liver (Rastogi *et al.*, 2001a). A significant increase in liver SOD activity occurred following AFB1 exposure of ducks (Barraud *et al.*, 2001).

One of the most important targets for mycotoxins is embryonic development. Since chicken embryo tissues contain high levels of PUFA, they are vulnerable to peroxidation, and oxidative stress caused by mycotoxins could be lethal. For example, aurofusarin increased late mortality of quail embryos (Dvorska *et al.*, 2001). Furthermore, contamination of the diet with T-2 toxin markedly decreased egg production and impaired hatchability (Tobias *et al.*, 1992). Confirmation of a possible association of this effect with oxidative stress came from data indicating that increased dietary vitamin E concentration during the first week of the experiment significantly decreased the number of infertile eggs and significantly improved the hatching percentage (Tobias *et al.*, 1992).

Increased lipid peroxidation as a consequence of mycotoxicoses

As illustrated in Table 1, OA has 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 of DNA, DNA adduct formation as well as LDH release were also used to confirm pro-oxidant properties of OA. Various *in vitro* and *in vivo* systems were also used including liver microsomes, phospholipid vesicles, primary cell cultures, whole organs and whole body.

T-2 toxin was also shown to have pro-oxidant properties (Table 2). 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 (Table 3). 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.

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 (Table 4). 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 (Karagezyan *et al.*, 1995; Ghedira-Chekir *et al.*, 1999) and citrinin (Ribeiro *et al.*, 1997).

Table 1. Stimulation of lipid peroxidation by ochratoxin A.

Mycotoxin	Tissue	Lipid peroxidation measurement	Protective effect of antioxidants	References
Ochratoxin A *in vitro*	Rat liver microsomes	TBARS ↑	-	Rahimtula *et al.*, 1988, Gautier *et al.*, 2001;
Ochratoxin A in feed	Rats	Ethane exhalation ↑	-	Rahimtula *et al.*, 1988
Ochratoxin A *in vitro*	Phospholipid vesicles	TBARS ↑, oxygen uptake ↑	-	Omar *et al.*, 1990
Ochratoxin A/analogs	*Bacillus brevis* bacteria	Free radical generation (EPR) ↑	Vitamin E	Hoehler *et al.*, 1996
Ochratoxin A in feed	Chicken liver	TBARS ↑	-	Hoehler and Marquardt, 1996; Hoehler *et al.*, 1997
Ochratoxin A	Vero cells in culture	TBARS ↑	SOD, catalase aspartame	Baudrimont *et al.*, 1997a; 1997b
Ochratoxin A in diet	Rat liver	TBARS ↑	-	Hoehler *et al.*, 1997
Ochratoxin A	A model oxidation system	Hydroxyl radical ↑, single-strand cleavage DNA ↑	-	Gillman *et al.*, 1999
Ochratoxin A	Primary cultures of astrocytes and neurones	TBARS ↑, LDH release ↑	- Retinol, ascorbic acid, vitamin E	Belmadani *et al.*, 1999 Grosse *et al.*, 1997
Ochratoxin A in feed	Mouse and rat kidney	DNA adduct formation ↑		
Ochratoxin A *in vitro*	Rat kidney microsomes	TBARS ↑	-	Gautier *et al.*, 2001

Table 2. Effect of T-2 toxin on lipid peroxidation.

Mycotoxin	Tissue	Lipid peroxidation measurement	Protective effect of antioxidants	References
T-2 in feed	Rat liver	TBARS ↑	Se, ascorbic acid, vitamin E	Tsuchida *et al.*, 1984; Suneja *et al.*, 1989; Schuster *et al.*, 1987; Rizzo *et al.*, 1994
T-2 in feed	Rat liver nuclei	TBARS ↑	-	Ahmed and Ram, 1986
T-2 in feed	Mouse liver	DNA fragmentation↑	CoQ10 and vitamin E	Atroshi *et al.*, 1997
T-2 in feed	Quail liver	TBARS ↑	Mycosorb®	Dvorska and Surai, 2001
T-2 in feed	Rat tissues	Conjugate diene ↑	-	Chang and Mar, 1988
T-2 in growth medium	Yeast	TBARS ↑	Vitamin E	Hoehler *et al.*, 1998

It is clear from these 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.

Protection against pro-oxidant effects of mycotoxins

The range of mycotoxins that can contaminate poultry feed and their different chemical structures make protection against mycotoxin-related toxicity a difficult task. There are various approaches to control or combat mycotoxin problems. The simplest strategy is based on the prevention of the formation of mycotoxins in feeds by special management programmes including storage at low moisture levels and prevention of grain damage during processing (Dawson, 2001). However, modern agronomic technology is not able to eliminate pre-harvest infection of susceptible crops by fungi (Wood, 1992). Therefore this strategy can only partially be effective; and in countries with warm and humid conditions, this strategy could be quite costly.

Other strategies based on microbial or thermal inactivation of toxins, physical separation of contaminated feedstuffs, irradiation, ammoniation and ozone degradation have not proved practical because they are either time-consuming or comparatively expensive (Dawson, 2001). In recent years, nutritional manipulation has been actively used to improve animal self-defence against mycotoxins or to decrease detrimental consequences of mycotoxin consumption.

Table 3. Stimulation of lipid peroxidation in rats by Aflatoxin B1.

Mycotoxin	Tissue	Lipid peroxidation measurement	Protective effect of antioxidants	References
Aflatoxin B1 in feed	Liver	TBARS ↑, conjugated dienes ↑	Se , vitamin E	Shen *et al.*, 1994
Aflatoxin B1	Cultured hepatocytes	TBARS ↑, LDH release ↑	SOD, catalase	Shen *et al.*, 1995
Aflatoxin B1 in diet	Liver	TBARS ↑	*Semecarpus anacardium* nut extract	Premalatha *et al.*, 1997
Aflatoxin B1	Cultured hepatocytes	TBARS ↑, ROS formation ↑	*Silvia miltorrhiza* extract	Liu *et al.*, 1999
Aflatoxin B1 (IP)*	Liver	TBARS ↑	Vitamin E, ternatin	Souza *et al.*, 1999
Aflatoxin B1 in feed	Liver	GSH-Px ↓	Se, vitamin E	Choi *et al.*, 1995
Aflatoxin B1 (IP)	Liver and kidney	TBARS ↑	Picroliv	Rastogi *et al.*, 2001a
Aflatoxin B1 (IP)	Liver	GSH, SOD, Catalase, GSH-Px ↓	-	Rastogi *et al.*, 2001b
Aflatoxin B1	Primary hepatocytes	TBARS ↑, GSH ↓, ROS generation ↑	-	Yang *et al.*, 2000
Aflatoxin B1	Liver	TBARS ↑	-	Ha and Kim, 1998
Aflatoxin B1	Liver	TBARS↑, GSH-Px↓, GSH-R↓	Melatonin	Meki *et al.*, 2001
Aflatoxin B1 *in vitro*	Model system	TBARS ↑	Vitamin E	Dvorska and Surai, 2001b

*IP = intraperitoneally

Since lipid peroxidation plays an important role in mycotoxin toxicity, a protective effect of antioxidants is expected (Galvano *et al.*, 2001). Indeed, as can be seen from data presented in Tables 1-4, protective effects against lipid peroxidation caused by mycotoxins were attributed to various antioxidant compounds including vitamins A and E, ascorbic acid, CoQ10, selenium, antioxidant enzymes as well as various plant extracts.

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 (Ledoux and Rottinghaus, 2000). Mycotoxins can be bound to the adsorbent and pass harmlessly through the digestive tract. Many compounds have been tested for adsorbent effects, however comparatively few have proven successful and still fewer (mainly bentonites, zeolites and alumosilicates) are used commercially (Devegowda *et al.*, 1998). 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. In addition to the various clays and zeolites, a yeast cell wall-derived glucomannan (Mycosorb®) has been shown to be effective against a wide range of mycotoxins (Devegowda *et al.*, 1998).

Mycotoxin binders can substantially improve the status of antioxidant systems in animals. This effect depends on the mycotoxin-binding activity of adsorbents. For example, inclusion of zeolite in the quail diet at 3% had a minor protective effect on the antioxidants in the quail liver and changes were not statistically significant (Figure 2; Dvorska and Surai, 2001a). Only the concentration of retinyl-linoleate in the liver of quail exposed to T-2 toxin simultaneously with zeolite was significantly higher compared with birds fed the diet containing T-2 toxin alone. These data indicate that zeolites alone were not effective in prevention of T-2 toxicity. These data are in agreement with observations of Kubena *et al.* (1990; 1998) indicating absence of protective effects of aluminosilicate sorbents against T-2 toxicosis. Superactivated charcoal (Edrington *et al.*, 1997) and inorganic sorbents (Bailey *et al.*, 1998) were also ineffective against T-2 toxicosis. Therefore, zeolite was probably unable to bind a substantial amount of T-2 toxin in the digestive tract; and as a result did not interfere with pro-oxidant properties of this mycotoxin.

In marked contrast, inclusion of yeast glucomannans (Mycosorb®) in T-2 toxin-containing diets fed quail significantly slowed the depletion of natural antioxidants and vitamin A in the liver (Figure 3). This protective effect can be attributed

Table 4. Effect of Fumonisin B1 and DON on lipid peroxidation.

Mycotoxin	Tissue	Lipid peroxidation measurement	Protective effect of antioxidants	References
Fumonisin B1	Rat liver nuclei	TBARS ↑, DNA strand breaks ↑	Catalase, mannitol	Sahu *et al.*, 1998
Fumonisin B1	Primary rat hepatocytes	TBARS ↑	Vitamin E	Abel and Gelderblom, 1998
Fumonisin B1	Rat liver	TBARS ↑	-	Abel and Gelderblom, 1998; Lemmer *et al.*, 1999; Gelderblom *et al.*, 2001
Fumonisin B1	Phosphatidylcholine bilayers	Rate of peroxidation ↑, free radical intermediate formation ↑, accelaration of chain formation ↑	-	Yin *et al.*, 1998
Fumonisin B1	Vero cells	TBARS ↑	-	Abado-Becognee *et al.*, 1998
DON in feed	Rat liver	TBARS ↑	Se, ascorbic acid, vitamin E	Rizzo *et al.*, 1994
DON in feed	Rat brain and spleen	GSH↓	-	Atroshi *et al.*, 1995
DON + 3-AcDON in feed	Mouse liver	TBARS ↑	-	Karppanen *et al.*, 1989

to the high adsorbent capability that esterified glucomannans have for T-2 (Dawson, 2001). 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.

Due to antioxidant depletion in the liver, susceptibility to lipid peroxidation increased more than 2-fold (Figure 4). Inclusion of zeolites in the diet did not prevent antioxidant depletion; and therefore susceptibility to lipid peroxidation in the liver was increased, showing no significant difference from the group fed the T-2 toxin treatment without an adsorbent additive. On the other hand, inclusion of Mycosorb® in the T-2-contaminated diet significantly decreased tissue susceptibility to lipid peroxidation in comparison to diets containing toxin only, although the inclusion of the Mycosorb® adsorbent material was unable to completely mitigate the powerful stimulating effect of T-2 toxin on lipid peroxidation.

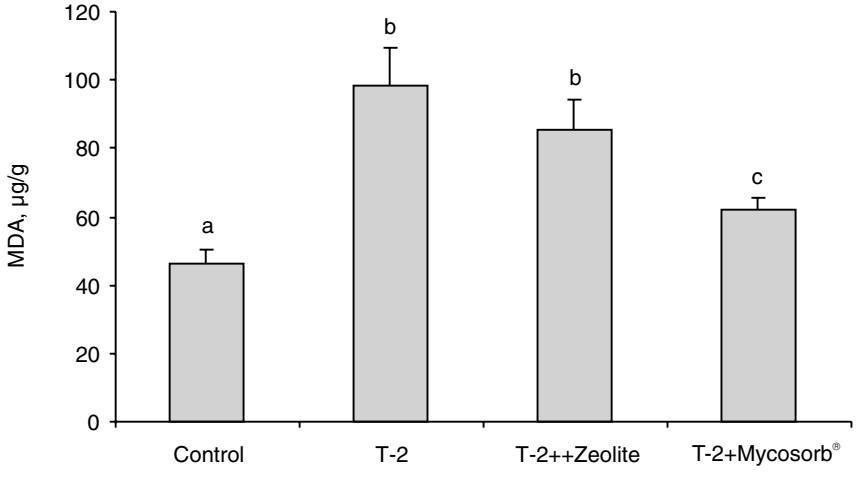

Figure 4. Lipid peroxidation in the quail liver. (Adapted from Dvorska and Surai, 2001).

Inclusion of Mycosorb® in the quail 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.

Conclusions

Stimulation of lipid peroxidation and consequent apoptosis are important mechanisms of the toxicity of various mycotoxins (Surai, 2002). Therefore, various natural antioxidants have been successfully tested as protective agents. However, using antioxidants alone it is impossible to prevent detrimental effects of mycotoxins on various metabolic processes in the body. Mycotoxin binders show promise in decreasing mycotoxin toxicity as well as in preventing damage to antioxidant systems. It is most likely that a combination of mycotoxin binders such as Mycosorb® with natural antioxidants such as organic selenium or vitamin E would be an effective approach to combat mycotoxicoses in future.

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