MYCOTOXINS AND OTHER CONTAMINANTS IN RABBIT FEEDS

Mézes M.

Department of Nutrition, Szent István University, Páter Károly u. 1., H-2103 Gödöllő, Hungary
Corresponding author: Mezes.Miklos@mkk.szie.hu

ABSTRACT

Animal-based food products derived from rabbit constitute a measurable portion of the human diet. Data from animal-production research demonstrate that the quality of these products is directly related to animal feeding practices. For that reason, the ingredients used in animal feed and their contamination with undesirable substances are fundamentally important for both the quality of the resulting food products and the potential human health impacts associated with the animal-based food-production chain. Alternatively, inclusion of feed ingredients, which are potentially toxic or contaminated with toxic substances, may result in a range of biological or toxicological effects in the production of animals. Animal feed ingredients that constitute complete feed products are derived from different raw materials, such as plant and animal origin, as well as pharmaceutical and industrial sources. Additionally, the contamination of feed materials from the environment would also be important as a potential hazard. The present review summarizes some of the toxic effects of some potentially hazardous ingredients of rabbit feed, such as mycotoxins (aflatoxins, ochratoxin, citritin, patulin, *Fusarium* mycotoxins – including trichothecenes, zearalenone, fumonisins, moniliformin and fusaric acid), fats and fatty acids, amino acids and coccidiostats. There are also some data about contaminants, such as heavy metals (arsenic, aluminium, cadmium, lead, mercury, molybdenum), and chlorinated dioxins and dibenzofurans.

Key words: Mycotoxins, Contaminants, Heavy metals, Dioxins, Toxicology, Rabbit.

MYCOTOXINS

Mycotoxins are invisible, highly corrosive, secondary metabolites of moulds which may persist in feed and even hay, when the moulds that produced them are no longer present (Scott, 1990). Nearly all of the mycotoxins are cytotoxic, disrupting various cellular structures such as membranes, and interfering with vital cellular processes such as protein, RNA and DNA synthesis (Guerre *et al.*, 2000). Mycotoxins destroy the tissues by oxidizing proteins and most of them have immunosuppressive effects. Some of them produce acute toxicity, evidenced by digestive disorders or dermatitis, but many more are carcinogenic, resulting in genetic mutations, or causing deformities in developing embryos. Mycotoxins can have very pervasive, yet sub-clinical, effects on the health of rabbits that more often go unnoticed. When the clinical symptoms of mycotoxin poisoning are observed, significant damage has occurred already. Improper harvesting, packaging and storage or prolonged shipping may enhance the potential for mould growth. Dirty harvesting, manufacturing/pelleting equipment and storage bins may contribute to mycotoxin contamination (Houssein and Brasel, 2001).

The symptoms are similar to more well-known ailments. Mycotoxins may cause fever (Cannon *et al.*, 1982), gastrointestinal problems, internal bleeding, haemorrhages or bruising, stomach ulcers (Aziz *et al.*, 1995), mouth sores, kidney, liver damages (Szilágyi *et al.,* 1994), central nervous system problems (Gabal *et al.*, 1986), immune-suppression (Richard *et al.*, 1991), tumour-genesis, eye, lung problems, hypertrophy of the adrenal cortex, reproductive organ problems (Szilágyi *et al.*, 1994), damaged heart muscle, tachycardia, skin problems (Fairhurst *et al.,* 1987), bone marrow and spleen problems (Niyo *et al.*, 1988), blood abnormalities (Mizutani *et al.,* 1997), rectal prolapses, and increased vascular fragility, respectively.

The poisoning may manifest chronic or acute episodes, depending on the amount of toxic feed ingested. The damage to organs is cumulative over a period of time. A high incidence of gastrointestinal upset and of diseases associated with depressed immune function (e.g. *Pasteurella*) may be clues for a mycotoxin problem exists (Richard *et al.*, 1991). There are some clinical signs, which may appear in rabbit, such as severe pain in the abdomen, while radiograph series may reveal gut shutdown, but no physical blockage, and sometimes severe bloating, hypothermia, several blood abnormalities, e.g. high urea and creatinine levels, calcium-phosphorus imbalance, abnormal levels of liver enzymes (AST, ALT, GGT), low hematocrite and RBC levels, ulcers in the mouth, stomach and oesophagus, feed refusal, weigh loss, presence of mucous in the faeces, rough hair coat, sometimes paralysis or twitching in hind limbs.

The European Commission has made recommendations (2006/576/EC) for the maximum level of several mycotoxins in complete diets (European Commission, 2006b) and regulation (2003/100/EC) for aflatoxins (European Commission, 2003), but only in some cases, particularly in rabbit feeds (Table 1).

Table 1: Recommended maximum amount of mycotoxins in complete feed

Mycotoxin	Maximum content ($mg\ kg^{-1}$ feed with 12% moisture)
Aflatoxin B_1	0.02
Ochratoxin A	5.00
Deoxynivalenol	5.00
Zearalenone	0.50
Fumonisin B_1+B_2	5.00

Aflatoxins

Rabbit is a highly susceptible species to aflatoxins produced by *Aspergillus* moulds. The LD_{50} of aflatoxin B_1 (AFB_1) in rabbits was determined as single oral dose 300 $\mu g\ kg^{-1}$ b.w. (FAO, 2000). However AFB_1 as low as 15 $\mu g\ kg^{-1}$ feed caused high level of morbidity and mortality (Makkar and Singh, 1991) and caused haemolytic anaemia, and strong cytotoxic effects were also observed (Verma and Mehta, 1998). Feeding diet naturally contaminated with 50 $\mu g\ kg^{-1}$ AFB_1 has caused lesions in the liver, absence of lobular architecture (Abdelhamid *et al.,* 2002). Bilirubin UDP-glucuronyltransferase activity was dramatically decreased, whereas cholestasis occurred as an effect of aflatoxicosis. An exponential dose-dependent increase in plasma bilirubin concentration was also observed. Both the simultaneous exponential increase in bilirubinemia associated to the reduced bilirubin UDP-glucuronyl-transferase activity and the absence of cholestasis suggested that the hyperbilirubinemia is more probably related to increased heme catabolism than to altered bile duct permeability (Guerre *et al.*, 1997). AFB_1 toxicity also caused damage of other tissues, such as kidney, testicles, brain and thyroids (Lakkawar *et al.*, 2004). The teratogenic effects of AFB_1 were described as enlarged eye sockets and enlarged liver of embryos (Wangikar *et al.*, 2005).

Beside the feed as the primary route of AFB_1 intake, inhalation of contaminated dust particles may result in high local exposure of the nasal mucosa. Larsson and Tjälve (2000) assessed the bio-activation and toxicity of AFB_1 in the nasal mucosa after intranasal administration of AFB_1, and also examined whether translocation of the mycotoxin occurs from the nasal mucosa to the brain along olfactory neurons. The data indicated that intranasal administration of AFB_1 resulted in formation of tissue-bound metabolites in subtentacular cells, in some cells of Bowman's glands, and in a population of neuronal cells in the olfactory mucosa, whereas in the respiratory nasal mucosa, there was selective bio-activation of AFB_1 in mucous cells. The data indicated materials transported in the olfactory nerves represent AFB_1 and/or some of its non-reactive metabolites. It is concluded that application of AFB_1 on the nasal mucosa results in high local bio-activation of the mycotoxin and translocation of AFB_1 and/or its metabolites to the olfactory bulb. The toxic effect of AFB_1 in the nasal mucosa is related to bio-activation of the mycotoxin. To our knowledge, there is no evidence that AFB_1 may induce tumours in olfactory bulbs. Thus, while tumours originating from nasal mucosa are frequently

found in livestock exposed to mouldy feed, no such evidence exists for forebrain neoplasm. Lack of CNS carcinogenesis is likely due to the inability of AFB_1 to pass from primary olfactory neurons to secondary or other neuronal connections in the olfactory system (Larsson and Tjälve, 2000).

As previously mentioned, AFB_1 is a potent hepatotoxic and hepato-carcinogenic mycotoxin that requires bio-activation to AFB_1-8,9-epoxide for activity (Essigmann *et al.,* 1982), which binds to DNA. Both endo- and exo-stereoisomers of AFB_1-8,9-epoxide exists, and although they are both produced in a variety of tissues, only exo-AFB_1-8,9-epoxide binds efficiently to DNA (Eaton and Gallagher, 1994). The International Agency for Research on Cancer has classified AFB_1 as a group I human carcinogen (IARC, 1993). Besides that, AFB_1 can contaminate respirable grain dust and thus the respiratory system is also a potential target for carcinogenesis. In addition to epoxidation, microsomal mono-oxygenases transform AFB_1 to the less toxic metabolites, aflatoxin M_1 (AFM_1) and aflatoxin Q_1 (AFQ_1). The rate of bio-transformation of AFB_1 depends on tissues, for instance values for AFM_1 formation in liver microsomes were greater than in lung, but the rate of AFQ_1 formation is the same in the above mentioned tissues (Daniels *et al*., 1990). Bio-activation-related toxicity of AFB_1 has also been observed in tracheal mucosa following intra-tracheal instillation of AFB_1 in rabbits (Coulombe *et al.,* 1986). These results indicate that besides liver, lung and trachea are capable to activate AFB_1 and that rabbit lung and tracheal microsomes contain high activity for this reaction (Daniels *et al*., 1990). Interestingly, some other toxic, and potentially carcinogenic constituents in the rabbit feed, like polycyclic aromatic hydrocarbons (PAH), such as α-naphthoflavone, induce the cytochrome P-450 system in the pulmonary and hepatic microsomes and consequently induces the detoxification of AFB_1, namely AFM_1 and AFQ_1 formation, and as well increases the DNA binding of AFB_1 (Daniels and Massey, 1992). Different rabbit lung cell types have different abilities to bio-activate AFB_1. Daniels *et al*., (1993) found that it was the highest in the microsomes of non-ciliated bronchiolar epithelial (Clara) cell-rich fraction.

Epoxide hydrolase and glutathione-S-transferase (GST) are both involved in hepatic detoxification of activated AFB_1, but the GST-catalyzed conjugation of glutathione to AFB_1-8,9-epoxides is thought to play the more important role in preventing epoxide binding to target macromolecules (Eaton and Gallagher, 1994). The glutathione-aflatoxin conjugate is transported from the cells with an ATP-dependent multidrug-resistance protein through an accelerated process (Loe *et al.,* 1997). Despite a preference for conjugating the more mutagenic AFB_1 exo-epoxide isomer, the relatively low capacity for GST-catalyzed detoxification of bio-activated AFB_1 in lung may be an important factor in the susceptibility of the lung to AFB_1 toxicity (Stewart *et al.,* 1996).

Ochratoxin

Ochratoxin A (OTA) is produced by several *Aspergillus* and *Penicillium* moulds. The International Agency for Research on Cancer has classified OTA as group 2B human carcinogen (IARC, 1993). In a study of Diwedi *et al.* (2004) OTA from *A. ochraceus* was given by gastric intubation to rabbits during 6-18 days of gestation at 0.025, 0.05 and 0.1 mg kg^{-1} body weight levels, respectively. Teratogenic effects were found at the 0.1 mg kg^{-1} dose group as a significant increase in the incidence of gross anomalies (wrist drop, rudimentary tail, knuckling of fetlock and agenesis of tail), skeletal (agenesis of caudal vertebrae, incomplete ossification of skull bones and wavy ribs), and soft tissue (internal hydro-cephalus, microphthalmia and kidney agenesis). The same embryo development abnormalities were observed by Wangikar *et al.* (2005). The number of live foetuses in the 0.1 mg kg^{-1} dose group was significantly lower than those of the 0.025 mg kg^{-1} dose group. The mean foetal weights and mean foetal crown to rump lengths of the 0.1 mg kg^{-1} OTA group were significantly lower than the 0.025 mg kg^{-1} dose group (Diwedi *et al*., 2004).

The effective transfer of OTA from the blood to the milk of lactating rabbit does and subsequently the exposure of their kits is also possible if the lactating rabbit does were fed a naturally-contaminated diet throughout the lactation period. However, approximately 99% of the plasma proteins bound to OTA (Chu, 1974), had the highest concentration of OTA accumulated in the body of the rabbit does were found in kidney followed by liver, mammary gland and muscle. A linear relationship was found

between the OTA concentrations in milk and in the plasma of the suckling kits, indicating an effective transfer of the toxin (Ferrufino-Guardia *et al.*, 2000). OTA is also extremely cytotoxic and may cause red blood cell haemolysis in rabbits (Zofair *et al.*, 1996). The proximal tubule of the kidney is the primary site targeted in OTA-induced nephrotoxicity (Suzuki *et al.*, 1975). The basolateral membrane organic anion transport pathway is involved in OTA accumulation in the renal cell (Groves *et al.*, 1998). However, at the same site, effective excretion of OTA was also found in an *in vitro* model system by Groves *et al.* (1999). They found that the basal-to-apical flux of OTA was increased with time in proximal tubular epithelial cells, and it was about eight-fold more than the apical-to-basal flux reabsorption. The above mentioned study also indicates that the secretion of OTA of rabbit renal proximal tubules is limited to the organic anion secretory pathway. In summary, the secretion of OTA represents a substantial avenue for removal of this mycotoxin from the systemic circulation (Groves *et al.*, 1999).

Citrinin

Rabbit production is important in tropical and subtropical agricultural systems (Cheeke, 1986), but some low-cost rabbit feed constituents, such as maize-milling waste might be infected with moulds, mainly *Aspergillus* and *Penicillium spp.*, and consequently might contain mycotoxins such as citrinin. Citrinin is a mycotoxin isolated originally from *Penicillium citrinum*. It has been found to be produced by a variety of other moulds, such as other *Penicillium* and *Aspergillus* species. Citrinin acts as a nephrotoxin in all farm animal species, including rabbit, but its acute toxicity varies (Bennett and Klich, 2003). The intraperitoneal LD_{50} value is calculated to be 19 mg kg^{-1} b.w. (Lakkawar *et al.,* 2004). Citrinin induces mitochondrial permeability pore opening (Da Lozzo *et al.*, 1998) and inhibits respiration by interfering with complex I of the respiratory chain (Chagas *et al.*, 1995). In the experiment of Hanika *et al.* (1984), a single oral dose of citrinin was given by gavages at dose levels of 20, 80 or 100 mg kg^{-1} b.w. The highest dose caused azotaemia and metabolic acidosis with haemo-concentration and hypokalaemia within one day, while as the effect of the two lower doses blood urea nitrogen and serum-creatinine levels increased, and creatinine clearance decreased indicating renal failure. Urine analysis indicated tubular dysfunction and necrosis with glucosuria, isosthenuria and cylindruria. Another possible consequence of low dose of citrinin toxicosis is the impairment of reproductive performance of rabbits in both genders as was found by Ajayi *et al.* (2005).

Patulin

Patulin is a mycotoxin produced by a variety of moulds, particularly *Aspergillus* and *Penicillum*. It is commonly found in moulded corn. It is not a particularly potent toxin, but some earlier studies have shown that it may be a carcinogen (Dickens and Jones, 1961), though studies have remained inconclusive. Effects of sublethal doses of patulin on the immune system were investigated in rabbits (Escuola *et al.*, 1988). They found significant suppression of the chemiluminescence response of peritoneal leucocytes. The mitogenic response to phorbol myristyl acetate, concanavalin A and, in particular, pokeweed mitogen was also depressed by patulin. This was parallel with decreasing serum immunoglobulin levels. The immune-suppressive effect of patulin is reversible and is probably due to interaction with cellular-free SH groups since the action of patulin can be circumvented, at least partially, by the prior administration of cysteine.

Fusarium mycotoxins

Fusarium fungi are commonly found in temperate climates and their mycotoxins are likely the most economically significant grain mycotoxins on a global basis (Wood, 1992). The numerous *Fusarium* mycotoxins are very diverse in chemical structure and characteristic mycotoxicosis. *Fusarium* moulds produce trichothecenes, such as T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS), monoacetoxy-scirpenol (MAS) and deoxynivalenol (DON), and also fumonisins, moniliformin, zearalenone (ZEN), fusaric acid, and verrucarin A (De Nus *et al.*, 1997). The main effect of *Fusarium* mycotoxins is to inhibit protein synthesis (trichotecenes), sphingolipid biosynthesis (fumonisins) and have nephrotoxic (moniliformin) and estrogenic effects (zearalenone). Clinical signs of *Fusarium* mycotoxicosis often

remain unclear because of the immune-suppressive effect of some *Fusarium* mycotoxins, mainly trichothecenes, which may cause decreased resistance to infectious diseases (Ueno, 1983).

Trichothecenes
Trichothecene mycotoxins are produced by *Fusarium* moulds mainly in fields and cause intoxication through consuming contaminated cereal crops in the compound feed (Placinta *et al*., 1999). About 150 different, but structurally related, trichothecenes have been chemically identified. The International Agency for Research on Cancer has classified trichothecenes as non carcinogens (IARC, 1993). Most of trichothecenes cause severe toxicosis in rabbit with relatively low LD_{50} values as shown in Table 2 (Wannemacher and Wiener, 1997).

Table 2: Relative acute toxicity of the most abundant trichothecene mycotoxins in rabbits

Trichothecene mycotoxin	Route of administration	LD_{50} (mg kg^{-1} body weight)
T-2 toxin	Intramuscular	1.1
T-2 toxin	Dermal (in dimethylsulfoxide)	10.0
4,15-diacetoxyscirpenol	Intravenous	1.0
Verrucarin A	Intravenous	0.54

However, most of the trichothecenes are partially metabolised by the microsomal xenobiotic transforming enzyme system. For instance, microsomal non-specific carboxyesterase produces C-4 acetyl residues of diacetoxyscirpenol, T-2 toxin, fusarenon-X and diacetylnivalenol. Some other trichothecenes, such as neosolaniol, HT-2 toxin, acetyl-T-2 toxin and tetraacetyl-nivalenol were unaffected by this hydrolysis (Ohta *et al*., 1978). As an effect of metabolism of trichothecenes mainly in the liver, their accumulation in rabbit meat is moderate or negligible. The rate of metabolism of trichothecenes, e.g. T-2 toxin, depends on the duration of exposure and decreases over a long period of time (Ványi *et al*., 1989). T-2 toxin causes lipid peroxidation in liver microsomes (Guerre *et al*., 2000), which also impairs the amount and/or activity of the xenobiotic transformation (Mézes *et al*., 1996). Guerre *et al*. (2000) found that a daily dose of 0.25 mg kg^{-1} b.w. of T-2 toxin results in decreased monooxygenase activity in rabbit liver. Total liver microsomal P450 content, and the activity of aminopyrine and benzphetamine *N*-demethylases, pentoxyresorufin *O*-depentylase, glutathione *S*-transferases accepting 1-chloro-2,4-dinitro-benzene and 1,2-dichloro-4-nitro-benzene as substrates, were decreased. By contrast, activity of ethylmorphine and erythromycin *N*-de-methylases, ethoxyresorufin and methoxyresorufin *O*-dealkylases, aniline hydroxylase, and UDP-glucuronyltransferase accepting *p*-nitrophenol as substrate, were unaffected. Gene expression of P450 1A1, 1A2, 2A1, and 2B4, but not P450 2C3 and 3A6, were also decreased. Beside those effects, microsomal oxidative damage was also proven by the significant increase of microsomal conjugated dienes, fluorescent substances, and malondialdehyde content. At a lower daily dose of T-2 toxin (0.1mg kg^{-1}), neither significant effects on drug metabolizing enzymes, nor microsomal oxidative damages were observed. Taken together, these results suggest that a short exposure of time to the mycotoxin would not be associated with significant changes in the normal metabolism of xenobiotics in the liver (Guerre *et al*., 2000).

Some nutritional effects such as deficiency or excess of some fat-soluble vitamins, namely vitamins A and E (Tutelyan and Kravchenko, 1988) increase the toxic effects of trichothecenes because of impairment of the activity of xenobiotic transforming enzyme system. For that reason, a decrease in the rate of conjugation and excretion of trichothecenes and their metabolites.
Trichothecenes inhibit cellular protein synthesis, a property which is probably the cause of many of the symptoms associated with trichothecene toxicoses. For instance, T-2 toxicosis results in hyper-aminoacidemia (Wannemacher and Dinterman, 1983), due to the inhibition of hepatic protein syn-thesis (Meloche and Smith, 1995). Subsequent elevations in blood tryptophan can result in increased concentrations of tryptophan in the brain. Tryptophan is the precursor of the neurotransmitter sero-tonin and the serotoninergic neurons are thought to be important mediators of behaviours, such as appetite, muscle coordination and sleep. Serotonin synthesis in the brain is poorly regulated and can be promoted by increased concentrations of tryptophan (Leathwood, 1987). Increased amount of brain serotonin is thought to cause loss of appetite and sleepiness.

Among the trichothecene mycotoxins, T-2 toxin caused feed refusal, a first symptom of toxicosis. At a single oral dose of 4 mg kg^{-1} b.w., T-2 toxin was lethal for rabbits within 48 h and proposed as LD$_{50}$ value for rabbits (Glávits *et al*., 1989). Faecal, caecotroph and urine toxin concentrations were related to toxin consumption (Fekete *et al*., 1989a). It is suggested that the high toxin level of the caecotroph can play role in the high sensitivity of the rabbit, and because of coprophagy, the animal will consume the toxin-containing caecotroph (Fekete *et al*., 1989b).

The main toxic effect of T-2 toxin is inhibition of protein synthesis which was proven by Ueno *et al.* (1973) when rabbit reticulocytes were treated with low concentrations of T-2 toxin and marked degradation of polyribosomes was observed. Feed containing sublethal amounts of T-2 toxin (12.5 and 25 mg kg^{-1} feed) caused emaciation, subacute catarrhal gastritis, necrosis of the lymphoid cells of the intestinal mucosa, depletion and necrosis in the lymphoid follicles of the ampulla ilei, spleen and lymph nodes (Fekete *et al*., 1989a). Niyo *et al.* (1988) described leukopenia, marginal anaemia, and increased number of morphologic changes in nucleated erythrocytes followed by a regenerative haematological response, centrilobular hepatocellular swelling portal and periportal fibrosis as an effect of T-2 toxicosis. Necrosis of lymphocytes, cells of the mononuclear phagocyte system, and myeloid haemocytogenesis were characteristics in most rabbits treated with T-2 toxin (Niyo *et al*., 1988; Glávits *et al*. 1989). T-2 toxin and its metabolites also decrease the spermiogenesis and libido in bucks, possibly because of the inhibition of conversion of pregnenolone to testosterone (Fenske és Fink-Gremmels, 1990).

Testing the effects of dermal exposure to T-2 mycotoxin showed moderate oedema and erythema at the site of T-2 mycotoxin exposure for two hours. T-2 toxin induces significant dermatitis and folliculitis characterized by infiltration of the superficial and deep dermis, epidermis, and follicular root sheaths by high numbers of heterophils, thickening of the superficial dermis due to separation of collagen fibres in oedema, and presence of intra-epidermal pustules. The cutaneous injury may be due to ischemia caused by microcirculatory failure (Yarom *et al*., 1987). Seven substituted trichothec-9-enes and six substituted trichothec-9-en-8-ones were tested for dermatotoxicity in rabbits, as estimated by induction of allopecia. Of these 13 variants, four showed the greatest toxicity: DAS, T-2 toxin, HT-2 toxin and fusarenon-X, especially the first two (Leonov *et al.,* 1990).

Toxic effects of deoxynivalenol (DON) or vomitoxin is very rare in rabbits. However, rabbit producers have been usually concerned with higher than normal deaths due to diarrhoea in rabbits. DON levels of commercial diets, particularly that contain wheat by-products contaminated with greater than 1 mg kg^{-1} DON has been blamed by some rabbit producers for this problem. The effects of DON on gastric emptying and intestinal propulsion in mice and rats and gastrointestinal myoelectrical activity in rats have been investigated (Fioramonti *et al*., 1993). Gastric emptying and intestinal transit were evaluated after gavages application of DON (50 to 1000 µg kg^{-1} b.w.). The myoelectrical activity of the antrum, duodenum and jejunum was measured 10 minutes after application. DON was found to inhibit gastric emptying in a dose-related manner. Intestinal propulsion was reduced only for the highest dose (1000 µg kg^{-1}). It was concluded that, in rodents, DON inhibits gastric emptying by inducing intestinal migrating motor complexes through a peripheral action at the serotonin-3 receptors. Pregnant does fed a DON contaminated diet showed marked weight loss, but no teratogenic effect was found (Khera *et al.,* 1986). In an *in vitro* erythrocyte model system extremely high doses of DON caused haemolysis (Rizzo *et al*., 1992), but that effect was not proven *in vivo* at naturally occurring contamination levels.

Zearalenone
Like the trichothecenes, zearalenone (F-2 toxin) is produced by *Fusarium* fungi. It is, however, chemically unrelated. Zearalenone (ZEN) has estrogenic properties (Koch, 1981); although it is not chemically an oestrogen and ZEN toxicity is more readily recognized, than trichothecene toxicity, because the symptoms are more specific. There are also data on its capability to induce adverse liver lesions with subsequent development of liver carcinoma (National Toxicology Program, USA, 1982),

however, the International Agency for Research on Cancer has classified ZEN as non carcinogen (IARC, 1993).

The effects of low (10 μg kg^{-1} b.w.) and high (100 μg kg^{-1} b.w.) oral doses of ZEN on some blood serum enzyme activities of AST, ALT, ALP, GGT, and total LDH of rabbits were studied by Čonková *et al.* (2001). Low doses resulted significant increase in ALP activity, while significant increases in activities of AST, ALT, AP, GGT, and LDH were observed, indicating possible liver toxicity due to chronic effects of the toxin. In rabbit bucks, ZEN impairs spermatogenesis and decreases libido, however only at extremely high dose levels (Fenske and Fink-Gremmels, 1990).

The *in vitro* reduction of ZEN by subcellular fractions from rabbit hepatocytes was investigated by Pompa *et al.* (1986). They found that in the presence of NADH, ZEN enhanced the reducing activity of the microsomal fraction. Furthermore, it was observed that hepatocytes produce α-zearalenol as the major and more uterotrophic metabolite. This means that rabbit has high sensitivity to the estrogenic effects of ZEN at dose levels of 0.1, 1 and 2 mg kg^{-1} b.w.

Fumonisins

Fumonisins are a family of mycotoxins that were first isolated in South Africa from cultures of *Fusarium verticillioides* (Gelderblom *et al.*, 1988), followed soon thereafter by elucidation of the structures of the prevalent isoforms fumonisin B$_1$ (FB$_1$) and B$_2$ (FB$_2$) (Bezuidenhout *et al.* 1988). It has been shown that the biochemical mode of action of the fumonisins is due to their chemical structure. They act as inhibitors of sphingolipid biosynthesis (Wang *et al.*, 1991) in most livestock species. In addition, the International Agency for Research on Cancer has classified fumonisins as a group 2B human carcinogen (IARC, 1993).

FB$_1$ was found to be nephrotoxic and hepatotoxic in rabbits (Gumprecht *et al.* 1995), and it has also been shown to exert deleterious effects on the haematopoietic organs (Mariscal-Quintanar *et al.* 1997). Histological examinations of liver showed centrolobular lipid infiltration, discrete cell necrosis, while nephrosis of the proximal tubuli was observed in the kidney. Lung oedema was found only in a low number of rabbits fed a FB$_1$ contaminated diet (Orova, 2003). Gumprecht *et al.* (2001) investigated the effects of FB$_1$ on the transport processes of the endothelial cells of lung capillaries of rabbits in an *in vitro* model system, but they did not detect measurable differences.

The teratogenic effect of FB$_1$ was also described using a dose of 300 mg day^{-1} for 14 days (Kovács *et al.*, 2003). In addition, changes of water distribution in the brain and lung of embryos in pregnant rabbit does fed the FB$_1$ contaminated diet was investigated using magnetic resonance spectroscopy by Orova (2003) and significant changes were found in both tissues as a consequence of FB$_1$ toxicosis even during intrauterine development.

In rabbit bucks, FB$_1$ (24.6 mg kg^{-1} feed) had no significant effect on testicular histometry. However, the weight distribution in the epididymides of the experimental animals demonstrated that the caput and caudal segments were significantly depressed in rabbits fed the highly contaminated diet. Results suggest that FB$_1$ may provide some protection against potential reduction in the sperm produced and stored in the testes and epididymides, but may elicit degenerations in the caput and caudal segments of the epididymides (Ogunlade *et al.*, 2006).

Moniliformin

Moniliformin is produced mainly by *Fusarium moniliforme*. It acts as an inhibitor of the tricarboxylic acid cycle in intermediary metabolism. This differs from the mode of action of the trichothecenes. No data are reported on the moniliformin toxicosis in rabbits, which might be due to its rare occurrence and low concentrations in feed (Thiel *et al.*, 1986).

Fusaric acid

Fusaric acid may act synergistically with the trichothecenes to reduce feed intake and cause lethargy in sensitive species (Smith, 1992), but there are no data reported in rabbit. *In vitro* studies support the

concept of a toxicological synergism between fusaric acid and the trichothecenes (Dowd, 1988) and Bacon *et al.* (1995) reported an interaction between fusaric acid and fumonisin B1. Fusaric acid, like the fumonisins and moniliformin, is produced mainly by *Fusarium moniliforme* (Burmeister *et al.*, 1985).

OTHER TOXIC FEED CONSTITUENTS AND CONTAMINANTS

Fats and fatty acids

The developmental toxicity of a 20% lipid emulsion that contains medium chain triglycerides to long chain containing lipid emulsion was investigated in rabbits (Henwood *et al.*, 1997). This emulsion was administered by intravenous infusion at dosages of 1 and 4.28 g lipid kg^{-1} b.w. once daily during organogenesis to assess the potential developmental toxicity. The results showed lower feed consumption of rabbit does but no other test article-related gross necropsy was found. However, embryo and foetal toxicity and skeletal abnormalities were found in rabbits received medium-chain triglycerides at the higher dose.

Fats, particularly which contain polyunsaturated fatty acids, are susceptible to rancidity. Vilas *et al.* (1976) found that feeding rancid fats decreases the growth rate and significantly increases the weight of intestinal epithelial layer, possibly because of inflammation in the intestine. Absorption of lipid hydro-peroxides from rancid fat is questionable. Some research support that reactive metabolites of the oxidised fats or fatty acids are reduced by the antioxidant enzymes, such as glutathione peroxidase, in the intestinal epithelial cells (Reddy *et al.*, 1974), while other studies have shown lipid hydroperoxides in the chylomicrons (Aw *et al.*, 1992) which reached the liver and caused impaired antioxidant defense of the liver cells (Mézes *et al.*, 1996), but severity of liver damage depends on the fat content of the diet (Slim *et al.,* 1995).

Amino acids

Excessive dietary methionine is known to exert the most toxic overall side effects as compared with all other protein amino acids (Benevenga and Steele, 1984). Liver plays a major and unique role in methionine metabolism and for that reason this organ appears to be exposed specifically to excessive methionine. A methionine enriched diet was reported to cause liver enlargement, fatty liver, and decreased liver ATP and glycogen levels (Hardwick *et al.*, 1970). The mechanisms of methionine hepatotoxicity are poorly understood, but several methionine metabolites are capable of injuring hepatocytes. For example, methanethiol, a product of the methionine transamination pathway, has been proposed to bind to cellular membranes and affect activities of the sulfhydryl-sensitive enzymes (Finkelstein and Bevenga, 1986). A growing body of evidence suggests that thiol compounds may be involved in free radical/lipid peroxidation processes (Munday, 1989). This was proven by Toborek *et al.* (1996) who found that long-term feeding of methionine enriched diet caused a significant increase in the amount of thiobarbituric acid reactive substances in liver that paralleled with increased activity of antioxidant enzymes and also induced atherosclerosis in rabbits (Toborek *et al.*, 1995).

Contaminants

An extensive, long-term analysis on feed toxicosis in rabbit farms in China was published by Gu *et al.* (2005). A total of 34,558 rabbits were poisoned and 8,551 died for a total mortality of 24.74%. Death rate depends on the toxic ingredients and the absorbed quantity, as well as the physiological condition of the rabbits. Among the total cases, more than 50% were caused by diet. In addition, a high percentage of abortion or stillbirth was found, mainly because of mycotoxins (Zilin, 2001). However, a relatively low number of cases, about 15%, were caused by mycotoxins. Plant toxins, such as gossypol (Zhengming, 1999), alkaloids, glycosides and oleandrism caused the same rate as mycotoxins, but with approximately 90% mortality. Contamination of feed ingredients with organic phosphorous insecticides also caused a high rate of mortality (Xianhua, 2002). Some additional cases

caused by other contaminants, such as nitrite (Zhaojiang, 2000) or urea (Zhengxian, 1998) with lower rate of mortality have been reported.

In another extensive study (Coleman and Tardiff, 1979), commercial rabbit feed samples originated from USA and Canada were collected and analyzed qualitatively and quantitatively for selected antibiotics, trace metals, pesticides (organophosphates and chlorinated hydrocarbons), natural agents, and polychlorinated biphenyls (PCBs). The results indicated that metals, chlorinated hydrocarbon pesticides, and polychlorinated biphenyls were continually present.

Coccidiostats

There is a general practice in rabbit feeding to use preventive veterinary drugs, such as coccidiostats. Rabbit is extremely sensitive to maduramycin (Tao, 2001) and flavomycin (Hanxiang, 1994). The well-known toxic coccidiostat in rabbits is narasin, which is a polyether carboxylic ionophore, generally used in poultry nutrition. Narasin poisoning in rabbits is mainly caused by cross-contamination of the compound feed. The cause of cross-contamination, as several studies have shown, is that the completely contamination-free production of premixes and compound feeds in the existing multi-product plants is impossible in practice (Strauch, 2003). Novilla *et al.* (1994) found that when rabbit bucks were treated with narasin by gavages with 30 or 100 mg kg^{-1} b.w., decreased locomotor activity, weakness in the extremities, and ataxia were observed 3 hours after administration. In addition, relaxation of the abdominal muscle, prone position, ptosis, decreased respiration, and unusual breathing were present. Narasin poisoning was also reported in rabbit warrens (Ősz *et al.*, 1988). Clinical symptoms started with a significant decrease in feed intake, followed by un-coordinated movement, weakness and flaccid paralysis of the extremities, especially in the posterior body half. Nervous symptoms (tonic-clonic convulsions, affecting the entire body, as well as torti-collis) were also observed. Death occurred one to four days after the onset of the clinical symptoms, frequently accompanied by significant malnutrition. In some acute cases, sudden deaths were also observed without any clinical signs. Enteritis and signs of circulatory disturbances were found in many cases. The histological lesions were characterised by moderate to severe Zenker's myofibrillar degeneration with lympho-histiocytic infiltration in the myocardium and skeletal muscle. Increases in serum enzyme activities (CPK, AST and ALT) showed a positive correlation with morphological damage of the muscular tissues. Narasin concentrations varying between 35 and 150 mg kg^{-1} feed caused acute intoxications and death. However, maternal toxicity was shown in rabbits at levels above 1 mg kg^{-1} b.w per day (EFSA, 2004).

Metals

Metal, particularly heavy metal toxicity in livestock production is a worldwide problem mainly in the industrialised countries. For that reason, the European Union regulated the maximum content of some heavy metals in complete feed as shown in Table 3 (European Commission, 2003, 2005a, 2005b)

Table 3: Maximum permitted content of some heavy metals in complete feed

Heavy metal	Maximum content (mg kg^{-1})	EU directive
Arsenic	2.00	2003/100/EC
Lead	5.00	2003/100/EC
Mercury	0.10	2005/8/EC
Cadmium	1.00	2005/87/EC

There are some data that lead content of kidneys and liver, cadmium contents of kidneys, liver, heart and muscles are several times higher in rabbits kept in industrial areas as compared to the tissue values of those rabbits which are kept far away from industrial areas (Krelowska-Kulas *et al.*, 2006). The same heavy metal accumulation was also found in wild rabbits (*Oryctolagus cuniculus* L.), the highest quantity of lead was found in muscle (3.81 mg kg^{-1}), while the highest cadmium (1.02 mg kg^{-1}) and mercury (0.08 mg kg^{-1}) values were found in kidney (Eira *et al.*, 2005).

The excess of metals may be harmful to the immune system, even slight exposure to heavy metals (lead, mercury and cadmium) alters immune-competence, although the exact mode of action is not

known, but the mentioned heavy metals impair the resistance against infections in rabbits (Fekete and Kellems, 2007).

Long-term cadmium toxicity caused splenic atrophy, and liver and kidney injury in rabbits (Stowe *et al.*, 1972). The oral lethal dose of cadmium was found to be 43 mg kg^{-1} b.w. (Fairchild *et al.*, 1977). Dietary cadmium load – e.g. carrots grown in cadmium contaminated soil – caused its accumulation in different tissues, mainly in the kidney (Bersényi *et al.*, 1999). Among the blood parameters, gamma-glutamyl-transferase and cholinesterase activities decreased significantly due to kidney and liver damage as an effect of feeding cadmium contaminated carrots. However, activity of alkaline phosphatase increased because of the pathological changes in the kidneys (Bersényi *et al.*, 1999). Long term cadmium exposure through the feed also impairs the reproductive ability of rabbit does because it causes morphological changes in the ovary (decreased volume of growing follicles and increased stroma and number of atretic follicles) also in the oviduct (oedematization), and in the uterus (oedematization); however, alterations were less in uterus in comparison with ovary and oviduct (Massányi *et al.*, 2007).

Metallothionein (MT) is a low-molecular-weight protein involved in detoxification of cadmium, however, results from the cadmium-heme assay showed that rabbits had low hepatic (2-10 μg g^{-1} liver) MT level (Henry *et al.*, 1994).

Aluminium also has been proposed to be an environmental factor that may contribute to some diseases, affecting several enzymes and other biomolecules and inducing free radical-mediated cytotoxicity. Aluminium also induces reproductive toxicity and exerts significant adverse effect on steroidogenesis (Guo *et al.*, 2005). The toxicity of aluminium chloride (34 mg kg^{-1} b.w.), given orally for six weeks daily on lipid peroxidation and enzyme activities in seminal plasma of male New Zealand white rabbits was investigated by Yousef *et al.* (2005). Results showed that aluminium toxicosis significantly decreased libido, ejaculate volume, sperm concentration, total sperm output, sperm motility, total motile sperm per ejaculate and packed sperm volume. Percentage of dead and abnormal sperm cells was also increased and the relative weights of testicles and epididymis were significantly decreased. Concentration of thiobarbituric acid-reactive substances (TBARS) was significantly increased in seminal plasma as compared to the control. Activities of glutathione *S*-transferase, AST, ALT and acid phosphatase were significantly decreased.

Beside feedstuffs and complete feeds, drinking water is a real source of toxic materials. Veeramchaneni *et al.* (2001) evaluated the effect of long-term (15 weeks) daily exposure of male rabbits to drinking water containing chemicals typical of ground water near hazardous waste sites. Ground water contained (mg l^{-1}) 7.75 arsenic, 1.75 chromium, 9.25 lead, 12.5 benzene, 3.75 chloroform, 8.5 phenol and 9.5 trichloroethylene, respectively. The control rabbit bucks consumed normal tap water. It was found that the ejaculatory capability decreased, but total spermatozoa per ejaculate and daily sperm production were unaffected by waste water consumption. However, treatment caused acrosomal dysgenesis and nuclear malformations. The baseline serum concentrations of LH were lower, but with borderline significance as compared to control bucks. However, testosterone secretion after exogenous human chorionic gonadotrophin (HCG) administration was low. Thus, exposure to drinking water pollutants caused subnormal mating ability, sperm quality, and Leydig cell function.

Other industrial by-products may also cause detrimental effects in rabbits when they are ingested with feed, including plastic residues, such as polyamides. For instance, *N*-butyl benzene-sulphonamide (NBBS), a plasticizer used commercially in the polymerization of polyamide compounds, is neurotoxic. Rabbits showed dose-dependent motor dysfunction characterized by limb splaying, hyperreflexia, hypertonia, gait impairment, and abnormal reflexes as an effect of long-term 4 to 12 months of NBBS intoxication. Histopathological changes consisted of intramedullary thickening of the ventral horn axons, random neuroaxonal spheroids confined to brain stem nuclei and spinal motor neurons, and swollen dendritic processes of spinal motor neurons were also observed (Strong *et al.*, 1991).

Polychlorinated dioxins and dibenzofurans

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are ubiquitous toxic contaminants originating mainly from thermal and incineration processes and representing potential risk for animal and human health. The International Agency for Research on Cancer has classified 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as the most potent dioxin congener as a group I human carcinogen (IARC, 1993). Various studies showed that environmental levels of this agent have decreased during the last decades (European Commission, 2000). In contrast to this trend, several cases of specific contamination have caused high PCDD and PCDF levels in feedstuffs. It is important to monitor the dioxin contamination of feed to avoid large-scale feed contamination and to decrease human exposure to dioxins. Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) were also found in some additives employed widely as binder and anti-caking agents in feedstuffs, such as kaolin, bentonite, and zeolite (Abad *et al*., 2002), also carriers of choline chloride, such as pine sawdust (Llerena *et al.,* 2003). The European Union has regulated (2006/13/EC) the maximum permitted amount of polychlorinated compounds in animal feed (European Commission 2006a) as is shown in Table 4.

Table 4: Maximum permitted amount of polychlorinated compounds in complete feed

Polychlorinated compounds	Maximum content (ng kg^{-1})
Dioxins (sum of PCDDs) and PCDFs expressed in WHO toxic equivalents, using the WHO-TEFC factors	0.75
Sum of dioxins (PCDDs and (PCDFs) and dioxin-like polychlorinated biphenyls (PCBs) expressed in WHO toxic equivalents, using WHO-TEFC factors	1.50

Compared to the feeds of other farm animal species, rabbit compound feeds are particularly contaminated with PCDDs and PCDFs (Table 5) as shown by an extensive survey in Italy (Cecil *et al*., 2004).

Table 5: Average PCDD/PCDFs concentrations expressed as WHO toxic equivalency factor in complete feed samples

PCDD/PCDF (ng kg^{-1})	Poultry	Cattle	Pig	Sheep	Rabbit
WHO-TEQ	0.022	0.035	0.019	0.021	0.319

Toxic effects of PCDDs and PCDFs in rabbits start with the so-called "wasting syndrome" because of feed refusal (Pohjanvirta and Tuomisto, 1994), later immune-depression and some neurologic effects are also manifested (Bursian, 2007). In the case of local skin contact with those reactive compounds, rabbits showed acne-like lesions on ears (Bursian, 2007). The acute oral LD_{50} value of polychlorinated biphenyls and dibenzo-*p*-dioxins in rabbit is relatively high 15 μg kg^{-1} b.w. (Safe, 1990).

REFERENCES

Abad E., Llerena J.J., Sauló J., Caixach J., Rivera J. 2002. Comprehensive study on dioxin contents in binder and anti-caking agent feed additives. *Chemosphere, 46, 1417-1421.*

Abdelhamid A.M., Ragab M.A., El-Shaieb A.F. 2002. The use of tafla or aluminosilicate for alleviating toxic effects of aflatoxin-contaminated diets of growing rabbits. *In Proc. 1st Conf. Animal & Fish Production, 2002, Mansoura, Egypt, 389-413.*

Ajayi F.O., Balogun O.O., Ovuru S.S., Mgbere O.O. 2005. Reproductive performance of rabbits fed maize-milling waste based diets. *Afr. J. Biotechnol., 4, 439-443.*

Aw T.Y., Williams M.W., Gray L. 1992. Absorption and lymphatic transport of peroxidised lipids by rat small intestine in vivo: role of mucosal GSH. *Am. J. Physiol., 262, G99-G106.*

Aziz N.H., El-Aziz A.M.A., Omran R.M.A. 1995. Effects of T-2 mycotoxin on histopathological changes in rabbits. *Biomed. Lett., 51, 271-281.*

Bacon C.W., Porter J. K., Norred W.P. 1995. Toxic interaction of fumonisin B1 and fusaric acid measured by injection into fertile chicken egg. *Mycopathologia, 129, 29-34.*

Benevenga N.J., Steele R.D. 1984. Adverse effects of excessive consumption of amino acids. *Annu. Rev. Nutr., 4, 157-179.*

Bennett J. W., Klich M. 2003. Mycotoxins. *Clin. Microbiol. Rev., 16, 497-516.*

Bersényi A., Fekete S., Hullár I., Kádár I., Szilágyi M., Glávits R., Kulcsár M., Mézes M., Zöldág L. 1999. Study of the soil-plant (carrot)-animal cycle on nutritive and hazardous minerals in a rabbit model. *Acta Vet. Hung., 47, 181-190.*

Bezuidenhout S.C., Gelderblom W.C.A., Gorst-Allman C.P., Horak R.M., Marasas W.F.O., Spiteller G., Vleggaar R. 1988. Structure elucidation of the fumonisins, mycotoxins from Fusarium moniliforme. *J. Chem. Soc. Chem. Commun., 1988, 743-745.*

Burmeister H.R., Grove M.D., Peterson D., Weisleder D., Plattner R.D. 1985. Isolation and characterization of two new fusaric acid analogs from Fusarium moniliforme NRRL 13,163. *Appl. Environ. Microbiol., 50, 311-320.*

Bursian S. 2007. Polychlorinated biphenyls, polybrominated biphenyls, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans. *In: Gupta, R.C. ed.: Veterinary Toxicology, Elsevier-Academic Press, Amsterdam-New York, 640-659.*

Cannon M., Cranston W.I., Hellon R.F., Townsend Y. 1982. Inhibition, by trichothecene antibiotics, of brain protein synthesis and fever in rabbits. *J. Physiol., 322, 447-455.*

Chagas GM, Oliveira MB, Campello AP, Klüppel ML. 1995. Mechanism of citrinin-induced dysfunction of mitochondria. III. Effects on renal cortical and liver mitochondrial swelling. *J. Appl. Toxicol., 15, 91-95.*

Cheeke P.R. 1986. Potentials of rabbit production in tropical and subtropical agricultural system. *J. Anim. Sci., 63, 1581-1586.*

Chu F.S. 1974. A comparative study of the interaction of ochratoxins with bovine serum albumin. *Biochem. Pharmacol., 23, 1105-1113.*

Ceci1 R., Diletti G., Torreti L., De Benedictis A., Scortichini G. 2004. Animal feed contamination by PCDDs-PCDFs in Italy in years 2002-2003. *Organohalogen Compounds, 66, 2041-2045.*

Coleman W.E., Tardiff R.G. 1979. Contaminant levels in animal feeds used for toxicity studies. *Arch. Environ. Contam. Toxicol., 8, 693-702.*

Coulombe R.A. Jr., Wilson D.W., Hsieh D.P.H., Plopper C.G., Serabjit-Singh C.J. 1986. Metabolism of aflatoxin B_1 in the upper airways of the rabbit: role of the nonciliated tracheal epithelial cell. *Cancer Res., 46, 4091-4096.*

Čonková E., Laciaková A., Pástorová B., Seidel H., Kováč, G. 2001. The effect of zearalenone on some enzymatic parameters in rabbits. *Toxicol. Lett., 121, 145-149.*

Da Lozzo E.J., Oliveira M.B., Carnieri E.G. 1998. Citrinin-induced mitochondrial permeability transition. *J. Biochem. Mol. Toxicol., 12, 291-297.*

Daniels J.M., Massey T.E. 1992. Modulation of aflatoxin B1 biotransformation in rabbit pulmonary and hepatic microsomes. *Toxicology, 74, 19-32.*

Daniels J.M., Liu L., Stewart R.K., Massey T.E. 1990. Biotransformation of aflatoxin B_1 in rabbit lung and liver microsomes. *Carcinogenesis, 11, 823-827.*

Daniels J.M., Matula T.I., Massey T.E. 1993. DNA binding and mutagenicity of aflatoxin B[1] catalyzed by isolated rabbit lung cells. *Carcinogenesis, 14, 1429-1434.*

De Nus M., Rombouts F., Notermans S. 1996. Fusarium molds and their mycotoxins. *J. Food Safety, 16, 15-58.*

Dickens F., Jones H.E.H. 1961. Carcinogenic activity of a series of reactive lactones and related substances. *Br. J. Cancer, 15, 85-100.*

Dowd P.F. 1988. Toxicological and biochemical interactions of the fungal metabolites fusaric acid and kojic acid with xenobiotics in Heliothis zea and Spodoptera frugiperda. *Pestic. Biochem. Physiol., 32, 123-131.*

Dwivedi P., Wangikar P.B., Sinha N. 2004. Teratogenic effects of ochratoxin A in rabbits. *World Rabbit Science, 12, 159-171.*

Eaton D.L., Gallagher E.P. 1994. Mechanisms of aflatoxin carcinogenesis. *Ann. Rev. Pharmacol. Toxicol., 34, 135-172.*

EFSA (European Food Safety Authority) 2004. Opinion of the Scientific Panel on Additives and Products or Substances Used in Animal Feed on a request from the Commission on the re-evaluation of efficacy and safety of the coccidiostat Monteban G100 in accordance with Article 9G of Council Directive 70/524/EEC. *The EFSA Journal, 90, 1-44.*

Eira C., Torres J., Vingada J., Miquel J. 2005. Concentration of some toxic elements in *Oryctolagus cuniculus* and in its intestinal cestode *Mosgovoyia ctenoides*, in Dunas de Mira (Portugal). *Sci. Total Environ., 346, 81-86.*

Escoula L., Thomsen M., Bourdiol D., Pipy B., Peuriere S., Roubinet F. 1988. Patulin immunotoxicology: Effect on phagocyte activation and the cellular and humoral immune system of mice and rabbits. *Int. J. Immunopharmacol., 10, 983-989.*

Essigmann J.M., Croy R.G., Bennett R.A., Wogan G.N. 1982. Metabolic activation of aflatoxin B_1: Patterns of DNA adduct formation, removal, and excretion in relation to carcinogenesis. *Drug Metab. Rev., 13, 581-602.*

European Commission 2000. Opinion of the Scientific Committee on Animal Nutrition (SCAN) on the dioxin contamination of feedingstuffs and their contribution to the contamination of food of animal origin, adopted on 6 November 2000, Brussels.

European Commission 2003. Commission directive 2003/100/EC amending Annex I to Directive 2002/32/EC of the European Parliament and the Council on undesirable substances in animal feed. *Official Journal L285, 33-37.*

European Commission 2005a. Commission directive 2005/8/EC amending Annex I to Directive 2002/32/EC of the European Parliament and the Council on undesirable substances in animal feed. *Official Journal L27, 44-45.*

European Commission 2005b. Commission directive 2005/87/EC amending Annex I to Directive 2002/32/EC of the European Parliament and the Council on undesirable substances in animal feed. *Official Journal L318, 19-24.*

European Commission 2006a. Commission directive 2006/13/EC amending Annexes I and II to Directive 2002/32/EC of the European Parliament and the Council on undesirable substances in animal feed as regards to dioxins and dioxin-like PCBs. *Official Journal L32, 44-53.*

European Commission 2006b. Commission Recommendation 2006/576/EC on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 toxin and fumonisin sin products intended for animal feeding. *Official Journal L229, 7-9.*

Fairchild E.J., Lewis R.J., Tatkin R.L. eds. 1977. Registry of toxic effects of chemical substances. *DHEW Publ. No. 78-104-B, 2, 526-534.*

Fairhurst S., Maxwell S.A., Seawin J.W., Swanston D.W. 1987. Skin effects of trichothecenes and their amelioration by decontamination. *Toxicology, 46, 307-319.*

FAO 2000. Aflatoxin research on grain in Asia – its problems and possible solutions. FAO Technical Report. *www.fao.org/impho/hilibrary/x0036e/x0036E.htm.*

Ferrufino-Guardia E.V., Tangni E.K., Larondelle Y., Ponchaut S. 2000. Transfer of ochratoxin A during lactation: expo-sure of suckling via the milk of rabbit does fed a naturally-contaminated feed. *Food Addit. Contam., 17, 167-175.*

Fekete S., Kellems R.O. 2007. Interrelationship of feeding with immunity and parasitic infection: a review. *Vet. Med. Czech., 52, 131-143.*

Fekete S., Tamás J., Ványi A., Glávits R., Bata, A. 1989a. Effect of T-2 toxin on feed intake, digestion and pathology of rabbits. *Lab. Anim. Sci., 39, 603-606.*

Fekete S., Tamás J., Ványi A., Glávits R., Bata A. 1989b. Effect of T-2 toxin fed in sublethal quantity on digestion in the rabbit (In Hungarian). *Magy. Allatorv. Lapja., 44, 739-740.*

Fenske M., Fink-Gremmels J. 1990. Effects of fungal metabolites on testosterone secretion in vitro. *Arch. Toxicol., 64, 72-75.*

Finkelstein A., Benevenga N.J. 1986. The effect of methanethoiol and methionine toxicity on the activities of cytochrome c oxidase and enzymes involved in protection from peroxidative damage. *J. Nutr., 116, 204-209.*

Fioramonti J., Dupuy C., Dupuy J., Bueno L. 1993. The mycotoxin, deoxynivalenol, delays gastric emptying through serotonin-3 receptors in rodents. *J. Pharmacol. Exp. Ther., 266, 1255-1260.*

Gabal M.A., Awad Y.L., Morcos M.B., Barakat A.M., Malik G. 1986. Fusariotoxicoses of farm animals and mycotoxic leucoencephalomalacia of the equine associated with the finding of trichothecenes in feedstuffs. *Vet. Hum. Toxicol., 28, 207-212.*

Gelderblom W.C.A., Jaskiewicz K., Marasas W.F.O., Thiel P.G., Horak M.J., Vleggaar R., Kriek N.P.J. 1988. Fumonisins - novel mycotoxins with cancer promoting activity produced by Fusarium moniliforme. *Appl. Environ. Microbiol., 54, 1806-1811.*

Glávits R., Ványi A., Fekete S., Tamás J. 1989. Acute toxicological experiment of T-2 toxin in rabbits. *Acta Vet. Hung., 37, 75-79.*

Groves C.E., Morales M.N., Wright S.H. 1998. Peritubular transport of ochratoxin A in rabbit renal proximal tubules. *J. Pharmacol. Exp. Ther., 284, 943-948.*

Groves C.E., Nowak G., Morales M. 1999. Ochratoxin A secretion in primary cultures of rabbit renal proximal tubule cells. *Am. Soc. Nephrol., 10, 13-20.*

Gu Z., Hao Y, Chen B., Ren W., Zhao C., Huang Y. 2005. Research and analysis on 211 cases of toxic disease of Chinese domestic rabbits. *Nature and Science (Peking), 3, 31-36.*

Guo C.H., Lin C.Y., Yeh M.S., Hsu G.S.W. 2005. Aluminium induced suppression of testosterone through nitric oxide production in male mice. *Environ. Toxicol. Pharmacol., 19, 33-40.*

Guerre P., Burgat V., Galtier P. 1997. Dose-related increase in liver heme catabolism during rabbit aflatoxicosis. *Toxicol. Lett., 92, 101-108.*

Guerre P., Eeckhoutte C., Burgat V., Galtier P. 2000. The effects of T-2 toxin exposure on liver drug metabolizing enzymes in rabbit. *Food Addit. Contam., 17, 1019-1026.*

Gumprecht L.A., Marcucci A., Weigel R.M., Vesonder R.F., Riley R.T., Showker J.L., Beasley V.R., Haschek W.M. 1995. Effects of intravenous fumonisin B1 in rabbits: Nephrotoxicity and sphingolipid alterations. *Nat. Toxins, 3, 395-403.*

Gumprecht L.A., Smith G.W., Constable P.C., Haschek W.M. 2001. Species and organ specificity of fumonisin-induced endothelial alteration: potential role in porcine pulmonary edema. *Toxicology, 160, 71-79.*

Hanika C., Carlton W.W., Boon G.D., Tuite J. 1984. Citrinin mycotoxicosis in the rabbit: Clinicopathological alterations. *Food Chem. Toxicol., 22, 999-1008.*

Hanxiang X. 1994. Prevention and treatment on flavomycin toxication. *J. Agric. Sci. Jiangsu Province, 3, 57-58.*

Hardwick D.F., Applegarth D.A. Cockroft D.M. 1970. Pathogenesis of methionine-induced toxicity. *Metabolism, 19, 381-390.*

Henry R.B., Liu J., Choudhuri S., Klaassen C.D. 1994. Species variation in hepatic metallothionein. *Toxicol. Lett., 74, 23-33.*

Henwood S., Wilson D., White R., Trimbo S. 1997. Developmental toxicity study in rats and rabbits administered an emulsion containing medium chain triglycerides as an alternative caloric source. *Fund. Appl. Toxicol., 40, 185-190.*

Houssein H.S., Brasel J.M. 2001. Toxicity, metabolism and impact of mycotoxins in humans and animals. *Toxicology, 167, 101-134.*

IARC 1993. Some naturally occuring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. *IARC Monogr. Eval. Carcinog. Risk Hum., 56, 1-599.*

IARC 1997. Polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans. *IARC Monogr. Eval. Carcinog. Risk Hum., 69, 1-631.*

Iyer R.S., Coles B.F., Raney K.D., Thier R., Guengerich F.P., Harris T.M. 1994. DNA adduction by the potent carcino-gen aflatoxin B1: mechanistic studies. *J. Am. Chem. Soc., 116, 1603-1609.*

Khera K.S., Whalen C., Angers G. 1986. A teratology study on vomitoxin (4-deoxynivalenol) in rabbits. *Food Chem. Toxicol., 24, 421-424.*

Koch H. 1981. Leitfaden der Medizinischen Mykologie. 3. Auflage. *VEB Gustav Fischer Verlag, Jena, Germany, p. 150.*

Kovács M., Romvári R., Orova Z., Kovács F., Horn P. 2003. Investigations on the foetotoxic effect of fumonisin B$_1$ in pig and rabbit (In Hungarian). *Acta Agr. Kaposváriensis, 7, 9-17.*

Krelowska-Kulas M., Kudelka W., Stalinski Z., Bieniek J. 2006. Content of metals in rabbit tissues. *Food Nahrung, 38, 393-396.*

Lakkawar A.W., Chattopadhyay S.K., Johri T.S. 2004. Experimental aflatoxin B1 toxicosis in young rabbits – A clinical and patho-anatomical study. *Slov. Vet. Res., 41, 73-81.*

Larsson P., Tjälve H. 2000. Intranasal instillation of aflatoxin B$_1$ in rats: Bioactivation in the nasal mucosa and neuronal transport to the olfactory bulb. *Toxicol. Sci., 55, 383-391.*

Leathwood P.D. 1987. Tryptophan availability and serotonin synthesis. *Proc. Nutr. Soc., 46, 143-146.*

Leonov A.N., Zotova E.V., Soboleva N.A., Kononenko G.P. 1990. Dermatotoxic activity of structurally related tricho-thecenes from Fusarium fungi (In Russian). *Dokl. Vsesoyuznoi Ordena Lenina i Ordena Trudovogo Krasnogo Znameni Akademii Sel'skokhozyaistvennykh Nauk imeni V.I. Lenina. No. 2, 52-55.*

Llerena J.J., Abad E., Caixach J., Rivera J. 2003. An episode of dioxin contamination in feedingstuff: the choline chloride case. *Chemosphere, 53, 679-683.*

Loe D.W., Stewart R.K., Massey T.E., Deeley R.G., Cole S.P. 1997. ATP-dependent transport of aflatoxin B1 and its glutathione conjugates by the product of the multidrug resistance protein (*MRP*) gene. *Mol. Pharmacol., 51, 1034-1041.*

Makkar H.P.S., Singh B. 1991. Aflatoxicosis in rabbits. *J. Appl. Rabbit Res., 14, 218-221.*

Mariscal-Quintanar M.G., Garcia-Escamilla R.M., Garcia-Escamilla N., Torres-Lopez J., Bautista-Ordonez J.A., Rosiles-Martinez R. 1997. Effect of ingesting Aspergillus flavus and Fusarium moniliforme on the cytology of bone marrow and blood and on serum albumin and globulin concentrations. *Veterinaria Mexico, 28, 75-81.*

Massányi P., Lukáč N., Uhrin V., Toman R., Pivko J., Rafay J., Forgács Zs., Somosy Z. 2007. Female reproductive toxicology of cadmium. *Acta Biol. Hung., 58, 287-299.*

Meloche J.L., Smith. T.K. 1995. Altered tissue amino acid metabolism in acute T-2 toxicosis. *Proc. Soc. Exper. Biol. Med., 210, 260-266.*

Mézes M., Virág Gy., Barta M, Abouzeid A.D. 1996. Effect of lipid peroxide loading on lipid peroxidation and on the glutathione and cytochrome systems in rabbits. *Acta Vet. Hung., 44, 443-450.*

Mizutani Y., Ito Y., Ohtsubo K. 1997. Inhibition of platelet aggregation in vitro by trichothecene nycotoxins (In Japanese). *Mycotoxins, 44, 41-44.*

Munday R. 1989. Toxicity of thiols and disulphides: involvement of free radical species. *Free Radic. Biol. Med., 7, 659-664.*

National Toxicology Program USA 1982. Technical report on the carcinogenesis bioassay of zearalenone in F 344/N rats and B6C3F1 mice (Feed Study). *NIH publ. N°83-1791, National Institute of Health, Bethedsa, USA, pp. 147.*

Niyo K. A., Richard J.L., Niyo Y., Tiffany L.H. 1988. Pathologic, hematologic, and serologic changes in rabbits given T-2 mycotoxin orally and exposed to aerosols of Aspergillus fumigatus conidia. *Am. J. Vet. Res., 49, 2151-2160.*

Novilla M.N., Owen N.V., Todd G.C. 1994. The comparative toxicology of narasin in laboratory animals. *Vet. Human Toxicol., 36, 318-323.*

Ogunlade J.T., Ewuola E.O., Gbore F.A., Bandyopadhyay R., Niezen J., G.N. Egbunike G.N. 2006. Testicular and epidi-dymal sperm reserves of rabbits fed fumonisin contaminated diets. *World Appl. Sci. J., 1, 35-38.*

Ohta M., Matsumoto H., Ishii K., Ueno Y. 1978. Metabolism of trichothecene mycotoxins. II. Substrate specificity of microsomal deacetylation of trichothecenes. *J. Biochem. (Tokyo), 84, 697-706.*

Orova Z. 2003. Investigations on the teratogenic effects of fumonisin B1 in swine and rabbit (In Hungarian). *Thesis, University of Kaposvár, Kaposvár, Hungary, pp. 23.*

Ősz M., Sályi G., Malik G., Dombi J., Bagó Gy. 1988. Narasin toxicosis in rabbit (In Hungarian). *Magy. Állatorv. Lapja, 43, 489-494.*

Placinta C.M., D'Mello J.P.F., Macdonald A.M.C. 1999. A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. *Anim. Feed Sci. Technol., 78, 21-37.*

Pohjanvirta R., Tuomisto J. 1994. Short-term toxicity of 2,3,7,8-tetra-chlorodibenzo-*p*-dioxin in laboratory animals: effects, mechanism, and animal models. *Pharmacol. Rev., 46, 483-549.*

Pompa G., Montesissa C., Di Lauro F.M., Fadini L. 1986. The metabolism of zearalenone in subcellular fractions from rabbit and hen hepatocytes and its estrogenic activity in rabbits. *Toxicology, 42, 69-75.*

Reddy K., Tappel A.L. 1974. Effect of dietary selenium and autooxidised lipids on the glutathione peroxidase system of gastrointestinal tract and other tissues in the rat. *J. Nutr., 104, 1069-1078.*

Richard J.L, Bray G.A., Ryan D.H. 1991. Mycotoxins as immunomodulators in animal systems. *Mycotoxins, cancer and health. Pennington Centre Nutrition Series, Vol. 1, 196-220.*

Rizzo A.F., Atroshi F., Hirvi T., Saloniemi H. 1992. The hemolytic activity of deoxynivalenol and T-2 toxin. *Nat. Toxins, 1, 106-110.*

Safe S. 1990. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurnas (PCDFs), and related compounds: environmental and mechanistic considerations which support the development the toxic equivalency factors (TEFs). *Crit. Rev. Toxicol., 21, 51-88.*

Scott P.M. 1990. Trichothecenes in grains. *Cereal Foods World, 35, 661-666.*

Slim R., Nicholas K.N., Toborek M., Biossonneault G.A., Hennig B. 1995. Hepatic susceptibility to oxidative stress in rabbits fed different high-fat diets. *In Proc. 95th Exp. Biol. Congr. Professional Research Scientist, 1995 August, Atlanta, USA, Part I, Abstr. 2454.*

Smith T.K. 1992. Recent advances in the understanding of Fusarium trichothecene mycotoxicoses. *J. Anim. Sci., 70, 3989-3993.*

Stewart R.K., Serabjit-Singh C.J., Massey T.E. 1996. Glutathione S-transferase-catalyzed conjugation of bioactivated afla-toxin B$_1$ in rabbit lung and liver. *Toxicol. Appl. Pharmacol., 140, 499-507.*

Stowe H.D., Wilson M., Goyer R.A. 1972. Clinical and morphological effects of oral cadmium toxicity in rabbits. *Arch. Pathol., 94, 389-397.*

Strauch W. 2003. Is contamination-free feed production realistic? *Feed Tech., 7(7), 23-25.*

Strong M.J., Garruto R.M., Wolff A.W., Chou S.M., Fox S.D., Yanagihara R. 1991. *N*-butyl benzenesulfonamide: a neurotoxic plasticizer inducing a spastic myelopathy in rabbits. *Acta Neuropathol., 81, 235-241.*

Suzuki S., Kozuka Y., Satoh T., Yamazaki M. 1975. Studies on the nephrotoxicity of ochratoxin A in rats. *Toxicol. Appl. Pharmacol., 34, 479-490.*

Szilágyi M., Fekete S., Huszenicza Gy., Albert M. 1994. Biochemical and physiological effects of long-term sublethal T-2 toxin feeding in rabbits. *Acta Biol. Hung., 45, 69-76.*

Tao F. 2001. Diagnosis on maduramicin toxication of rabbit. *China Vet. Sci., 3, 37-38.*

Thiel P.G., Gelderbloom W.C.A., Marasas W.F.O., Nelson P.E., Wilson T.M. 1986. Natural occurrence of moniliformin and fusarin C in corn screenings known to be hepatocarcinogenic in rats. *J. Agr. Food Chem., 34, 773-781.*

Toborek M., Kopieczna-Grzebieniak E., Drózdz M., Wieczorek M. 1995. Increased lipid peroxidation as a mechanism of methionine-induced atherosclerosis in rabbits. *Atherosclerosis, 115, 217-224.*

Toborek M., Kopieczna-Grzebieniak E., Drózdz M., Wieczorek M. 1996. Increased lipid peroxidation and antioxidant activity in methionine-induced hepatitis in rabbits. *Nutrition, 12, 534-537.*

Tutelyan V.A., Kravchenko L.V. 1988. Nutrition effects on T-2 toxin toxicity. *Proc. Jpn. Assoc. Mycotoxicol., Suppl. 1, 107-108.*

Ueno Y. Nakajima M., Sakai K., Ishii K., Sato N., Shimada N. 1973. Comparative toxicology of trichothec mycotoxins: Inhibition of protein synthesis in animal cells. *J. Biochem., 74, 285-296.*

Ványi A., Glávits R., Bata A., Fekete S., Tamás J. 1989. The pathological effects, metabolism and excretion of T-2 toxin in rabbits. *J. Appl. Rabbit Res., 12, 194-200.*

Veeramachaneni D.N.R., Palmer J.S., Amann R.P., Korner W., Kogevinas M., Bourguignon J.-P. 2001. Long-term effects on male reproduction of early exposure to common chemical contaminants in drinking water. *Acta Pathol. Microbiol. Immunol. Scand., 109, S320-S329.*

Verma R.J., Mehta D.N. 1998. Occurrence of hemolytic anemia during aflatoxicosis. *Ind. J. Environ. Toxicol., 8, 5-7.*

Vilas N.N., Bell R.R., Draper H.H. 1976. Influence of dietary peroxides, selenium and vitamin E on glutathione peroxidase of the gastrointestinal tract. *J. Nutr., 106, 589-596.*

Wang E., Norred W.P., Bacon C.W., Riley R.T., Merrill A.H. 1991. Inhibition of sphingolipid biosynthesis by fumonisins: implications for diseases associated with Fusarium moniliforme. *J. Biol. Chem., 266, 14486-14492.*

Wangikar P.B., Dwivedi P., Sinha N., Sharma A.K., Telang A.G. 2005. Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and aflatoxin B_1 with special reference to microscopic effects. *Toxicology, 215, 37-47.*

Wannemacher R.W., Dinterman R.E.1983. Plasma amino acid changes in guinea pigs injected with T-2 mycotoxin. *Fed. Proc., 42, 625 (Abstr.).*

Wannemacher R.W., Wiener S.L. 1997. Trichothecene mycotoxins. In: *Medical Aspects of Chemical and Biological Wearforce. Textbook of Military Medicine, Burden Institute, Washington DC, USA.*

Wood G.E. 1992. Mycotoxins in foods and feeds in the United States. *J. Anim. Sci., 70, 3941-3948.*

Xianhua P. 2002. Prevention and treatment on dichlorphos toxication of rabbit. *Report Agric. Sci. Technol., 5, 22-24.*

Yarom R., Bergmann F., Yagen B 1987. Cutaneous injury by topical T-2 toxin: Involvement of microvessels and mast cells. *Toxicon, 25, 167-174.*

Yousef M.I., Salem M.H., Kamel K.I., Hassan G.A., El-Noufty F.D. 2003. Influence of ascorbic acid supplementation on the haematological and clinical biochemistry parameters of male rabbits exposed to aflatoxin B_1. *J. Environ. Sci. Health, 38B, 193-209.*

Yousef M.I., El-Morsy A.M.A, Hassan M.S. 2005. Aluminium-induced deterioration in reproductive performance and seminal plasma biochemistry of male rabbits: Protective role of ascorbic acid. *Toxicology, 215, 97-107.*

Zhaojiang J. 2000. Study on nitrite toxication of rabbit. *J. Husbandry Vet., 2, 46-50.*

Zhengxian L. 1998. Urea toxication of rabbit. *J. China Vet., 9, 31-32.*

Zilin G. 2001. Study on dead fetus of rabbit. *J. Feed Indust., 10, 43-45.*

Zofair S.M., Mathew S., Verma R.J. 1996. Ochratoxin induced hemolysis in rabbits. *Ind. J. Exp. Biol., 34, 592-593.*

Zhengming X. 1999. Study on cottonseed toxication of rabbit. *J. China Vet., 7, 53-55.*