MycoToxins and Other Contaminants in Rabbit Feeds

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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 (Guerré 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**

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Maximum content (mg kg⁻¹ feed with 12% moisture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B₁</td>
<td>0.02</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>5.00</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>5.00</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>0.50</td>
</tr>
<tr>
<td>Fumonisin B₁+B₂</td>
<td>5.00</td>
</tr>
</tbody>
</table>

**Aflatoxins**

Rabbit is a highly susceptible species to aflatoxins produced by *Aspergillus* moulds. The LD₅₀ of aflatoxin B₁ (AFB₁) in rabbits was determined as single oral dose 300 µg kg⁻¹ b.w. (FAO, 2000). However AFB₁ as low as 15 µg 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 µg kg⁻¹ AFB₁ has caused lesions in the liver, absence of lobular architecture (Abdelhamid *et al*., 2002). Bilirubin UDP-glucurononyltransferase 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). AFB₁ toxicity also caused damage of other tissues, such as kidney, testicles, brain and thyroids (Lakkawar *et al*., 2004). The teratogenic effects of AFB₁ were described as enlarged eye sockets and enlarged liver of embryos (Wangikar *et al*., 2005).

Beside the feed as the primary route of AFB₁ 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 AFB₁ in the nasal mucosa after intranasal administration of AFB₁, 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 AFB₁ 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 AFB₁ in mucous cells. The data indicated materials transported in the olfactory nerves represent AFB₁ and/or some of its non-reactive metabolites. It is concluded that application of AFB₁ on the nasal mucosa results in high local bio-activation of the mycotoxin and translocation of AFB₁ and/or its metabolites to the olfactory bulb. The toxic effect of AFB₁ in the nasal mucosa is related to bio-activation of the mycotoxin. To our knowledge, there is no evidence that AFB₁ 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\textsubscript{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\textsubscript{1} is a potent hepatotoxic and hepato-carcinogenic mycotoxin that requires bio-activation to AFB\textsubscript{1}-8,9-epoxide for activity (Essigmann et al., 1982), which binds to DNA. Both endo- and exo-stereoisomers of AFB\textsubscript{1}-8,9-epoxide exist, and although they are both produced in a variety of tissues, only exo-AFB\textsubscript{1}-8,9-epoxide binds efficiently to DNA (Eaton and Gallagher, 1994). The International Agency for Research on Cancer has classified AFB\textsubscript{1} as a group I human carcinogen (IARC, 1993). Besides that, AFB\textsubscript{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\textsubscript{1} to the less toxic metabolites, aflatoxin M\textsubscript{1} (AFM\textsubscript{1}) and aflatoxin Q\textsubscript{1} (AFQ\textsubscript{1}). The rate of bio-transformation of AFB\textsubscript{1} depends on tissues, for instance values for AFM\textsubscript{1} formation in liver microsomes were greater than in lung, but the rate of AFQ\textsubscript{1} formation is the same in the above mentioned tissues (Daniels et al., 1990). Bio-activation-related toxicity of AFB\textsubscript{1} has also been observed in tracheal mucosa following intra-tracheal instillation of AFB\textsubscript{1} in rabbits (Coulombe et al., 1986). These results indicate that besides liver, lung and trachea are capable to activate AFB\textsubscript{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\textsubscript{1}, namely AFM\textsubscript{1} and AFQ\textsubscript{1}, formation, and as well increases the DNA binding of AFB\textsubscript{1} (Daniels and Massey, 1992). Different rabbit lung cell types have different abilities to bio-activate AFB\textsubscript{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\textsubscript{1}, but the GST-catalyzed conjugation of glutathione to AFB\textsubscript{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\textsubscript{1} exo-epoxide isomer, the relatively low capacity for GST-catalyzed detoxification of bio-activated AFB\textsubscript{1} in lung may be an important factor in the susceptibility of the lung to AFB\textsubscript{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\textsuperscript{-1} body weight levels, respectively. Teratogenic effects were found at the 0.1 mg kg\textsuperscript{-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 hydrocephalus, 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\textsuperscript{-1} dose group was significantly lower than those of the 0.025 mg kg\textsuperscript{-1} dose group. The mean foetal weights and mean foetal crown to rump lengths of the 0.1 mg kg\textsuperscript{-1} OTA group were significantly lower than the 0.025 mg kg\textsuperscript{-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 Klisch, 2003). The intraperitoneal LD50 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 haemococoncentration 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 Penicillium. 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 trichotheccenes, such as T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS), monoaetoxy-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 (trichotheccenes), 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 \textit{Fusarium} mycotoxins, mainly trichothecenes, which may cause decreased resistance to infectious diseases (Ueno, 1983).

\textit{Trichothecenes}  
Trichothecene mycotoxins are produced by \textit{Fusarium} moulds mainly in fields and cause intoxication through consuming contaminated cereal crops in the compound feed (Placinta \textit{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).

\begin{table}[h]  
\centering  
\begin{tabular}{lll}  
<table>
<thead>
<tr>
<th>Trichothecene mycotoxin</th>
<th>Route of administration</th>
<th>LD$_{50}$ (mg kg$^{-1}$ body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-2 toxin</td>
<td>Intramuscular</td>
<td>1.1</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>Dermal (in dimethylsulfoxide)</td>
<td>10.0</td>
</tr>
<tr>
<td>4,15-diacetoxyscirpenol</td>
<td>Intravenous</td>
<td>1.0</td>
</tr>
<tr>
<td>Verrucarin A</td>
<td>Intravenous</td>
<td>0.54</td>
</tr>
</tbody>
</table>
\end{tabular}  
\caption{Relative acute toxicity of the most abundant trichothecene mycotoxins in rabbits}  
\end{table}

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 diacetyl-nivalenol. Some other trichothecenes, such as neosolaniol, HT-2 toxin, acetyl-T-2 toxin and tetraacetyl-nivalenol were unaffected by this hydrolysis (Ohta \textit{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 \textit{et al}., 1989). T-2 toxin causes lipid peroxidation in liver microsomes (Guerre \textit{et al}., 2000), which also impairs the amount and/or activity of the xenobiotic transformation (Mézes \textit{et al}., 1996). Guerre \textit{et al}. (2000) found that a daily dose of 0.25 mg kg$^{-1}$ b.w. of T-2 toxin results in decreased monoxygenase 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 \textit{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 synthesis (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 serotonin and the serotonergic 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 alopecia. 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 trichotheccenes, 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 centrilobular 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 trichotheccenes (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 oleandrin 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 (Zhao Jiang, 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 uncoordinated 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 torticollis) 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>
<thead>
<tr>
<th>Heavy metal</th>
<th>Maximum content (mg kg(^{-1}))</th>
<th>EU directive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>2.00</td>
<td>2003/100/EC</td>
</tr>
<tr>
<td>Lead</td>
<td>5.00</td>
<td>2003/100/EC</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.10</td>
<td>2005/87/EC</td>
</tr>
<tr>
<td>Cadmium</td>
<td>1.00</td>
<td>2005/87/EC</td>
</tr>
</tbody>
</table>

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

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>
<thead>
<tr>
<th>PCDD/PCDF (ng kg(^{-1})) WHO-TEQ</th>
<th>Poultry</th>
<th>Cattle</th>
<th>Pig</th>
<th>Sheep</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.022</td>
<td>0.035</td>
<td>0.019</td>
<td>0.021</td>
<td>0.319</td>
<td></td>
</tr>
</tbody>
</table>

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).

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


