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Fumonisins and their effects on animal health - a brief review

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ABSTRACT

Fumonisins are mycotoxins produced by certain *Fusarium* moulds, principally *Fusarium moniliforme* a worldwide corn contaminant. It is already well known that fumonisins are associated with various animal toxicosis and carcinomas, including leukoencephalomalacia (ELEM), porcine pulmonary oedema (PPE), hepatotoxicosis, hepatocarcinomas, nephrotoxicosis and immunosuppressive effects. Among fumonisin isomers FB_1 is a predominant molecular form and major toxicant. It has been found that FB_1 disrupts the sphingolipid metabolism on many types of cells and tissues through the inhibition of ceramide synthase, a key enzyme in *de novo* sphingolipid biosynthesis and reacylation of free sphingosine. The second mechanism of toxicity involves changes in polyunsaturated fatty acid and disruption of membrane phospholipids. Different toxic effects of FB_1 were evaluated in many kinds of animal, such as Equidae, swine, rat, mice rabbit, chicken, non-human primates and others. Exposure assessment, legislative and efficient detoxification is needed to be done in order to avoid unwanted consequences and economic losses.

Key words: fumonisins, *Fusarium moniliforme*, animal toxicosis, ceramide synthase, phospholipids

Introduction

Fumonisins are mycotoxins produced principally by *Fusarium moniliforme* (Sheldon), which is a worldwide contaminant of corn and corn-products consumed by humans and animals. Their pathogenic effects include fatal diseases in farm and laboratory animals, such as equine

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leukoencephalomalacia (ELEM) (WILSON et al., 1991), porcine pulmonary oedema (PPE) (HARRISON et al., 1990), hepatotoxicity, hepatocarcinogenicity (GELDERBLOM et al., 1991, GELDERBLOM et al., 2001) and nephrotoxicity (NORRED et al., 1996; BUCCI et al., 1998).

Fumonisins in home-grown corn have been associated with an elevated risk of human oesophageal cancer in South Africa and China (RHEEDER et al., 1992; CHU and LI, 1994).

The toxicology, natural occurrence of fumonisins in corn, and implications for animal health will be discussed in this review.

Historical background

Toxic effects of fumonisins were observed at the beginning of the last century as sporadic fatal conditions in horses and related Equidae. The disease was named leukoencephalomalacia (ELEM) (BUTLER, 1902). The incriminated causative agent of ELEM was corn feed infected with species of the genus *Fusarium* (WILSON and MARONPOT, 1971), in particular, *F. moniliforme* (Fig. 1.).

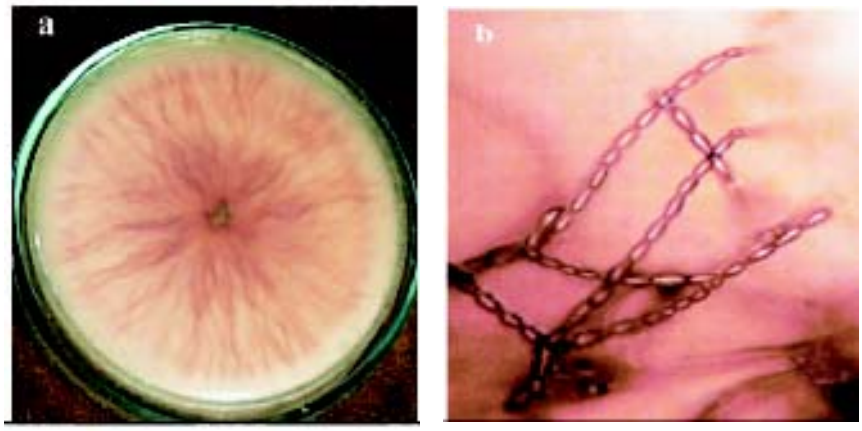

Fig. 1. *Fusarium moniliforme* on Potato-dextrose agar (a), and under the microscope (b); ×500

This was not a useful observation since *F. moniliforme* produces a range of mycotoxins: fusaric acid, moniliformin and fusarin C (BRUCKNER et al., 1989). The real cause of ELEM was discovered in 1988 (Programme on Mycotoxins and Experimental Carcinogenesis-PROMEC) in South Africa, when the two toxic metabolites (FB_1-macrofusin, FB_2) were originally isolated from *F. moniliforme* strain (BEZUIDENHOUT et al., 1988). Later, the same compounds were isolated from *Alternaria* sp. (CHEN et al., 1992). An additional investigation line in South Africa studied causes of the high prevalence of oesophageal cancer in Transkei, and the difference between the fungal flora from oesophageal and non-oesophageal areas was established (MARASAS et al., 1980). Isolated fungal strains of *F. moniliforme* were found to be toxic and carcinogenic to rats (GELDERBLOM et al., 1988). Experimental feeding of horses by using purified FB_1 caused typical ELEM symptoms with necrotic brain lesions (KELLERMAN et al., 1990). Other studies on animal species established lung oedema in pigs (HASCHEK et al., 1992; OSWEILER et al., 1992), and liver cancer in rats (GELDERBLOM et al., 1991). However, fumonisins did not produce oesophageal cancer in any animal species tested, including primates (FINCHAM et al., 1992). The nephrotoxic effect of fumonisins was later investigated, but they were earlier reported in horses, pigs, sheep, rats, mice and rabbits (BUCCI et al., 1998).

Chemical characteristics of fumonisins

Fumonisins (B_1, B_2, A_1, A_2) were first reported by BEZUIDENHOUT et al. (1988). Cultures of *F. moniliforme* MRC 826 on corn were used to isolate the fumonisins. A group of investigators independently isolated the compound macrofusin from a strain of *F. moniliforme* isolated from corn associated with ELEM in New Caledonia (LAURENT et al., 1989). Macrofusin and FB_1 were found to be identical. A few years later FB_3 and FB_4 were also isolated and characterized (CAWOOD et al., 1991; PLATTNER et al., 1992). The B series of fumonisins (Fig. 2) are esters of 2-amino-12, 16-dimethyl-14, 15-dihydroxyecosane, and propan-1, 2, 3-tricarboxylic acid. Fumonisin B_1 has hydroxyl groups at C-3, C-5, C-10. Fumonisins B_2 and B_3 are isomers with hydroxyl groups at C-3, C-5 and C-3, C-10. Fumonisin B_4 has one less hydroxyl group than FB_2 and FB_3. Fumonisins A_1 and A_2 are N-acetyl

derivates of FB_1 and FB_2. Fumonisin C_1 has also been isolated and differs from FB_1 in a lack of methyl group at C-1, which is characteristic of the other fumonisins. MACKENZIE et al. (1998) isolated a new fumonisin from liquid culture of *F. moniliforme* NRRL 13613, which was named iso-FB_1 and differs from FB_1 only in the placement of hydroxyl group at C-4 instead of C-5.

	R_1	R_2	R3
FA_1	OH	OH	CH_2CO
FA_2	H	OH	CH_2CO
FB_1	OH	OH	H
FB_2	H	OH	H
FB_3	OH	H	H
FB_4	H	H	H
FC_1	OH	OH	H

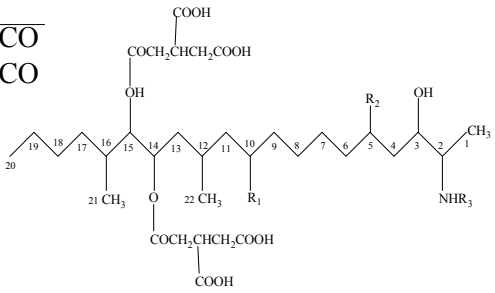

Fig. 2. Chemical structure of fumonisins

In comparison with other fumonisins, FB_1 is the most polar compound. In polar solvent, FB_1 exists as zwitterion because of carboxylic groups, which can have positive and negative charges, and also because of existing free primer amine. Small changes of pH can produce alterations in the structure of tricarboxylic esters and it can cause interactions between active groups. However, the chemical structure of fumonisins point to the existence of a high number of stereoisomers. Among them, FB_1 is a predominant molecular form produced by *F. moniliforme*; FB_2 and FB_3 appear to be active as FB_1 although they occur in lower concentrations; FA_1 and FA_2 lack the toxicity and promotion activity of FB_1 (GELDERBLOM et al., 1992). Fumonisins are potent competitive inhibitors of the ceramide synthase-key enzyme in sphingolipid biosynthesis (WANG et al., 1991). In rat primary hepatocytes, tricarboxylic moiety is required for inhibition of this enzyme. The presence of an amino group appears not to be a requisite for activity (MERRILL et al., 1996).

The fumonisins mechanism of action in the biological system

It has been found that FB_1 disrupts the sphingolipid metabolism in many types of cells and tissues, including hepatocytes, neurons and renal cells (RILEY et al., 1996). Eucaryotic cells synthesize a large amount of molecules, which build sphingolipids - important structure units of membrane and regulators of many cell functions. In the animal cell, synthesis of sphingolipids occurs on the endoplasmic reticulum by condensation of serine and palmytoil-CoA. The biosynthesis of glicosphingolipids and sphingomyelin is located on the lumen site of the Golgi complex membrane. Complex sphingolipides are degraded within lysosomes endosomes and plasmatic membrane and free sphingoid bases can be found in a cytosol (MCKEE and MCKEE, 1996). The biosynthesis of sphingolipids (*de novo*) begins with condensation of serine and palmytiol-CoA by serine palmitoyltransferase, a pyridoxal-5'-phosphate-dependent

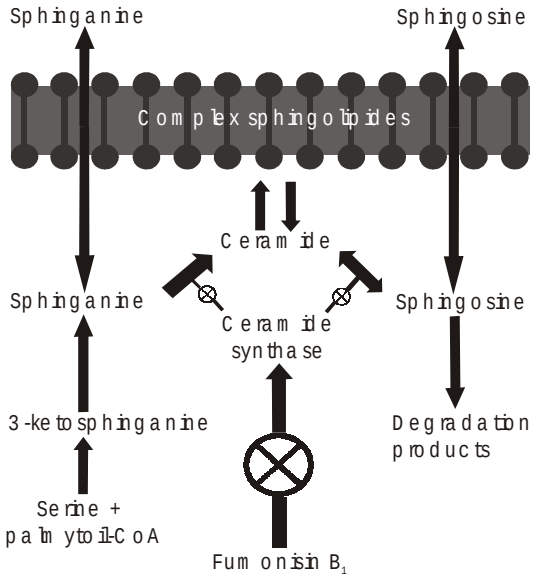

Fig. 3. Pathway of sphingolipid biosynthesis and sphingosine turnover

enzyme (Fig. 3). The resulting 3-keto-sphinganine is subsequently reduced to sphinganine using NADPH. Sphinganine is acylated to dihydroceramides (N-acyl sphinganines) by enzyme ceramide synthase (sphinganine-N-acyltransferase) using various fatty acyl-CoAs. Ceramides are then converted to glycosphingolipides and sphingomyelin (RILEY et al., 1996). Sphingolipid turnover involves the hydrolysis of complex sphingolipids to ceramides, and then to sphingosine, which is either reacylated or phosphorilated and cleaved to a fatty aldehyde and ethanolamine phosphate, which is incorporated into phosphatidyl-ethanolamine (SMITH and MERILL, 1995). Sphingosine normally occurs in low concentrations in cells and regulates several important pathways as a lipid second messenger (NORRED, 1993). Because of the similarity of the polyhydric alcohol moiety of fumonisins to sphingosine, fumonisins can interfere with sphingolipid biosynthesis or sphingosine turnover. Fumonisins (B_1, B_2, B_3 and hydrolysed B_1) potently disrupt the acylation of sphinganine and sphingosine (Fig. 3) by inhibition of ceramide synthase. The inhibition of this enzyme causes an increase in intracellular sphinganine concentration. In primary rat hepatocytes, the IC_{50} for inhibition of serine incorporation into sphingosine is approximately 0. 1 mM for FB_1 and FB_2 (WANG et al., 1991; MERRILL et al., 1993). In cultured renal cells (LLC-PK_1), the IC_{50} for inhibition of *de novo* sphingosine biosynthesis is approximately 20 mm FB_1 (YOO et al., 1992).

In microsomal preparations from cultured neurons, inhibition of ceramide synthase is competitive with respect to sphingiod bases and fatty-acyl-CoA. Sphingomyelin biosynthesis is approximately 10 times more sensitive to inhibition of ceramide synthase by FB_1 than glycolipide biosynthesis (MERRILL et al., 1993; RILEY et al., 1996). Sphingolipids have a complex role in cell function. Fumonisin disruption of normal sphingolipid metabolism affects a great number of processes. Accumulation of sphingoid bases can cause growth inhibition of normal cells and cytotoxicity. They can inhibit protein kinase-C, activate phospholipase D, activate or inhibit enzymes involved in lipid signalling pathways, inhibit Na^+/K^+ ATP-ase, and induce dephosphorylation of retinoblastoma protein. All of these processes may increase cancer risk via loss of regulation of differentiation, apoptosis and lipid mediators that control cell proliferation (MERRILL et al.,

1996; RILEY et al., 1996; LEE et al., 1998). Inhibition of ceramide synthase *in vivo* resulted in accumulation of free sphinganine in liver, lung and kidney. Sphinganine, as a hydrophobic compound, can cross cell membranes and occur in blood, and in urine if the kidneys were affected (HANNUN et al., 1991; RILEY et al., 1996). Therefore, serum samples from horses dosed with FB_1 containing feed were analysed for changes in sphingosine and sphinganine levels. The levels of both compounds increased in proportion to FB_1 intake (WANG et al., 1992). Similar effects on serum and tissue (lung, liver, kidney) levels of sphingosine and sphinganine in response to FB_1 were established in rats and swine. Elevation of the ratio of sphinganine level to sphingosine (Sa/So) is the most sensitive indicator of *in vivo* effects of fumonisins, and was proposed as a biomarker for exposure to fumonisins (RILEY et al., 1993a; RILEY et al., 1994).

The mechanism of action of FB_1 also involves changes in poly-unsaturated fatty acid and phospholipid pools. In rat liver, FB_1 induced changes in phospholipids and their fatty acid composition which affected many cell functions that can contribute to its toxicity and carcinogenicity. Changes in the concentrations of specific polyunsaturated fatty acids were attributed to disruption of D6 desaturase and cyclo-oxygenase metabolic pathways (GELDERBLOM et al., 2001; ANONYMOUS, 2001). Fumonisin B_1 altered in absolute and relative amounts of phosphatidylcholine and ethanolamine and in degree of saturation of their fatty acids in microsomal, mitochondrial, plasma, nuclear cell membranes and membranes associated with hepatic nodules. These biochemical effects lead to increased lipid peroxidation, mitoinhibition, hepatotoxicity, increased expression of hepatocyte growth factor, transforming growth factor (TGF-a, TGF-b1), and proto-oncogene c-*myc*, alterations in retinoblastoma pathway, deregulation of cell cycle control by over-expression of cyclin D1 protein, and finally, tumour promotion and hepatocarcinogenicity (GELDERBLOM et al., 2001; RAMLJAK et al., 2000; ANONYMOUS, 2001).

Fumonisin B_1 is non-mutagenic in the *Salmonella* mutagenicity test (GELDERBLOM and SNYMAN, 1991), and is not genotoxic in the *in vivo* or *in vitro* DNA repair assays in rat primary hepatocytes (NORRED et al., 1992; GELDERBLOM et al., 1994), but it is citotoxic and genotoxic to RK13 cells (RUMORA et al., 2002). Also, FB_1 caused chromosomal aberrations in primary

rat hepatocytes (KNASMULLER et al., 1997), and it is mitogen in cultured Swiss 3T3 murine fibroblasts (SCHROEDER et al., 1994).

Toxic effects of fumonisins on animals

Horse. ELEM has been reported since the nineteenth century. In 1901 more than 600 horses died in an outbreak in North Carolina (BUTLER, 1902), and more than 5000 horses died between 1934 and 1935 in Iowa and Illinois (BIESTER et al., 1940; SCHWARTE et al., 1937). Synonyms for ELEM include mouldy corn poisoning, blind staggers, corn stalk disease, and circling disease. Association of the disease with ingestion of corn contaminated with *F. moniliforme*-major producer of fumonisins was recognized. ELEM commonly presents itself as an epizootic. Attack rates range from 14 to 25% and 62 to 100%. The majority of outbreaks occur in the fall through early spring. The course of the disease depends on the concentrations of fumonisins in feed, the amount of feed consumed, and the tolerance of the individual horse to fumonisin (McCUE, 1989; UHLINGER, 1991). Fumonisins cause neurological disease in horses, indicating multifocal CNS involvement. The initial clinical signs include depression; inappetence after a period of eating contaminated feed, horses become lethargic and, as neurotoxic effects become apparent, develop uncoordinated movement (blind staggers) (KELLERMAN et al., 1990; ROSS et al., 1993; WILSON et al., 1992). Animals can die 7 hours or even a few years after the first clinical symptoms occur (KRIEK et al., 1981). UHLINGER (1997) reviewed the histopathology of ELEM caused by fumonisins. Histopathology is characterised with lesions in the CNS, described as malacia degeneration or liquefactive necrosis involving the subcortical white matter of one or both cerebral hemispheres. Similar lesions may develop in the brain stem, cerebellum, and spinal cord. Haemorrhages involve the CNS pleurae and abdominal cavities. The periphery of the lesions is often marked by perivascular haemorrhage and oedema. The other organ affected is the liver, which often shows a mild swelling with a colour change to yellow-brown and with irregular white foci and nodules scattered throughout the parenchyma. Liver lesions may be seen with fibrosis of the centrilobular area in severe cases. Hepatic lesions are less common than CNS lesions in horses exposed to fumonisis.

Renal lesions, described variously as hydropic degeneration, nephrosis, nephropathy or individual cell necrosis, were reported incidentally (KELLERMAN et al., 1990). These observations are supported by experiments using fumonisins in added culture material (B_1, B_2, B_3), or using a purified compound (KELLERMAN et al., 1990; RILEY et al., 1997; ROSS et al., 1993, 1994; WILSON et al., 1992). THIEL et al. (1991) reported that the cumulative threshold of FB_1 is 10.8 ppm, while WILSON et al. (1992) suggested that this limit for horses is much lower (8 ppm). In addition, SCHUMACHER et al. (1995) studied the role of *F. moniliforme* cultures in duodenitis and proximal jejunitis in horses, and observed a copious reflux of gastric juice, which might be related to FB_1 ingestion.

Swine. Two outbreaks of disease in swine were described in the U.S.A. during 1988 and 1989 (HARRISON et al., 1990; BANE et al., 1992; OSWEILER et al., 1992). Autopsy established pulmonary oedema and hydrothorax, with the thoracic cavities being filled with yellow liquid, and was added to a "mystery swine disease". Samples of feed were taken from outbreaks and analysed for fumonisins; FB_1 was found in a range of 20-330 mg/kg. The disease, called porcine pulmonary oedema syndrome (PPE), was linked with outbreaks of ELEM (ROSS et al., 1990). Studies on swine included feed naturally contaminated with fumonisins or by application of purified toxins (MOTELIN et al., 1994; COLVIN et al., 1993; HASCHEK et al., 1992). Animals fed with high levels of FB_1 died from pulmonary oedema, while those fed with lower levels of FB_1 had sub-acute hepetotoxicosis. Animals receiving pure toxin were more likely to develop pulmonary oedema, while those fed with naturally contaminated feed had both lesions. Three young swine that were given feed with FB_1 in a range of from 100 to 190 mg/kg developed nodular hyperplasia of the liver and changes in distal oesophageal mucose. These variations can be explained by differences between acute and chronic poisoning, or by the different action of fumonisins when applied either in pure form or together with other metabolites in naturally contaminated material, including known synergistic effects between FB_1 and fusaric acid (BACON et al., 1995). Swine intubated with *F. moniliforme* culture material, besides developing lung oedema and liver necrosis, also developed mild to moderate renal tubular necrosis. In swine fed with purified FB_1 (1.5 mg/kg daily, 64 mg/swine total), or feed

contaminated with 20 ppm FB_1 and 7 ppm FB_2 (34 and 44 mg/kg total dose), Sa/So ratios in the kidney, liver and lung were markedly elevated after only 5 days, with the greatest elevation in the kidney. Lesions were produced in liver and lung, but not the kidney, in some animals (COLVIN et al., 1993; HASCHEK et al., 1992). Sa/So ratio was suggested (RILEY et al., 1993a) as an early biomarker for exposure of swine to FB_1. Pancreas, heart and oesophagus can also be the target organs of fumonisins (HARRISON et al., 1990; CASTEEL et al., 1993; CASTEEL et al., 1994). CONSTABLE et al. (2000) reported that in swine ingestion of FB_1 (20 mg/kg body mass) produce negative inotropic and chronotropic effect and a decrease in the mechanical efficiency of the left ventricle. Cardiovascular effects are consistent with fumonisin-induced sphingosine-mediated L-type Ca^{2+} channel blockade, suggesting that PE in swine fed fumonisin is primarily due to acute left-sided heart failure, rather than increased vascular permeability.

Rat, mice, rabbit. Before the discovery of fumonisins, rats were dosed with feed infected with *F. moniliforme* related to outbreaks of ELEM (WILSON et al., 1985; VOSS et al., 1989). These studies established the development of hepatic nodules, adenofibrosis, hepatocellular carcinoma, and cholangiocarcinoma. Other work reported by the PROMEC group shows that the fumonisins are hepatotoxins and carcinogens in the rat. Culture material from *F. moniliforme* fed to rats produced micro and macronodular cirrhosis, cholangiofibrosis and primary hepatocellular carcinomas (GELDERBLOM et al., 1991). Several potential mechanisms of fumonisin hepatotoxicity and carcinogenicity are interruption of sphingolipid biosynthesis, disruption of phospholipid metabolism, fatty acid accumulation and cell proliferation, oxidative stress and lipid peroxidation, oxidative DNA damage and peroxisome proliferation (GELDERBLOM et al., 1996; ANONYMOUS, 2001). MARTINEZ-LARRANGA et al. (1996) reported that FB_1 is a co-inducer of microsomal and peroxisomal fatty acid oxidation, emphasizing that these changes may contribute to FB_1 hepatocarcinogenicity in rats. They established that FB_1, at dose levels of 0.25 and 2.5 mg/kg once a day for 6 days, induces the cytochromes P4501A1 and P4504A1, which are responsible for w-hidroxylation of fatty acids, and induction of peroxisomal fatty acid b-oxidation enzyme system. Fumonisin B_1 also produced peroxisome proliferation. In male Fischer

rats fed diets containing FB_1 for 21 days, the concentration of cyclin D1 protein in liver was increased with no increase of cyclin D1 mRNA levels. Accumulation of cyclin D1 resulted from protein stabilisation associated with activation of protein kinase B (Akt), followed by inhibition of glycogen synthase kinase 3b (GSK-3b). This caused increase in cyclin-dependent kinase 4 (Cdk4) which resulted in hyperphosphorilation of retinoblastoma protein and an alteration in cell cycle progression (G_1/S) of rat hepatocytes, which suggested the mechanism of FB_1 carcinogenicity (RAMLJAK et al., 2000). Recently, a two-year FB_1 carcinogenicity study on rats and mice was carried out (HOWARD et al., 2001) Male (n=48) and female (n=48) F344 rats and $B6C3F_1$ mice were exposed to FB_1 (96% pure) in feed containing different concentrations. After treatment there was clear evidence of carcinogenic activity of FB_1 in male F344 rats based on an incidence of renal tubule adenomas and carcinomas in 9/48 and 15/48 at 50 and 150 ppm, respectively. There was no evidence of carcinogenic activity of FB_1 in female F344 rats with doses as high as 100 ppm. Hepatocellular adenomas and carcinomas were induced by FB_1 in the female mice, occurring in 5/47, 3/48, 1/48, 19/47 and 39/45 female mice at 0, 5, 15, 50, and 80 ppm FB_1, respectively. There was no evidence of carcinogenic activity of FB_1 in male $B6C3F_1$ mice exposed to up to 150 ppm FB_1 in diet. Apoptosis, increased cell proliferation and hyperplasia of renal tubule epithelium in exposed rats were observed, particularly in the group that developed renal tubule neoplasms. In mice exposed to the higher concentrations of FB_1, males and females had increased incidences of hepatocellular hypertrophy, while females had increased incidences of hepatocellular apoptosis. An earlier series of short- and long-term studies in mice and rats reviled that liver and kidney were the major target organs of FB_1 toxicity, with individual cell necrosis. Variations among species were also observed; e. g. $B6C3F_1$ mice were more resistant to FB_1 than F344 rats. Female mice developed hepatotoxicity when fed 81 ppm, while doses at 27 ppm or less had no effect on female and male mice. In contrast, both sexes of rats given 9 ppm or more developed renal toxicity. Hepatotoxicity was not produced even in the 27 and 81 ppm groups (VOSS et al., 1995). Results showed that FB_1 is nephrotoxic at doses that do not cause liver toxicity. Short-term studies (HOWARD et al., 1995; BUCCI et al.,

1998) showed that mice fed 100-500 ppm FB_1 indicated minimal to mild hepatotoxicity in all females and males in the 250 and 500 ppm groups. Nephrotoxicity was not produced in these mice. In rat, biliary hyperplasia and hepatocellular degeneration were present in females given 163, 234 or 484 ppm and in males given 234 and 484 ppm. Degeneration of renal tubular epithelium was produced at doses between 99 and 484 ppm in males and between 163 and 484 ppm in females. Necrosis of hepatocytes and renal tubule cells were found to be apoptotic. One study in male Sprague-Dawley rats (LIM et al., 1996) showed increased cell proliferation occurring in the oesophagus following a single i. v. dose FB_1 (1.25 mg/kg body mass), suggesting that the oesophagus is a specific target for fumonisin toxicity. Pregnant mice fed culture material containing fumonisins were shown to have lowered body mass, increased morbidity and mortality in a dose-response reaction (GROSS et al., 1994). Other observations were liver damage and ascites and a reduction in the number of live offspring. In a study to determine the FB_1 target organs in rabbit (GUMPRECHT et al., 1995), animals were treated intravenously with 0.15 to 1 mg FB_1/kg for 4 or 5 daily doses. After multiple doses, the rabbits were lethargic and anorectic, with decreased urine production and biochemical indications of liver and kidney injury. The most striking lesions were in the kidney and consisted of severe necrosis of proximal tubule epithelium in the outer strip of the medulla and within the cortex. Glomeruli appeared normal. Liver lesions included individual cell necrosis, hepatocellular swelling and bile stasis. Sa/So ratios were increased in liver kidney, muscle, serum and urine, but not brain.

Chickens. Given the economic importance of chickens and their dependence on corn-based feeds, investigations of fumonisin effects on these animals were carried out. In two studies, day-old broiler chicks were fed with FB_1 ranging from 0-400 mg /kg for 21 days and 300 mg/kg for 2 weeks (BROWN et al., 1992). Body mass gain was greatly reduced and hepatic necrosis, biliary hyperplasia, and thymic cortical atrophy was noted. Increased Sa/So ratios were established in young chicks treated with culture material containing FB_1 (WEIBKING et al., 1993). When the broiler chicks were fed with FB_1 and FB_2 they showed abnormal erythrocyte formation and lymphocyte cytotoxic effects (DOMBRINK-KURTZMANN et al., 1993).

Chicken embryos exposed to FB_1 showed a mortality of 100% when dosed at 100 mM. Pathological changes were found in liver, kidney, heart, lungs, muscular-skeletal system, intestines, testis and brain (JAVED et al., 1993). The effects of FB_1 on chicken peritoneal macrophages showed a breakdown of the nucleus with increasing numbers in a dose-response manner (CHATTERJEE et al., 1995). Ingestion of feed with 25% corn cultures contaminated with *F. moniliforme*, or feed containing 61 ppm of FB_1 and 14 ppm of FB_2, manifested as depressed antibody responses to sheep red blood cells and *Brucella abortus* (MARIJANOVIĆ et al., 1991; QURESHI et al., 1995; BONDY and PESTKA, 2000). Peritoneal macrophage numbers and phagocytic ability were also compromised (QURESHI et al., 1995).

Primates. Primate studies were undertaken primarily to investigate possible development of oesophageal cancer, which occurrs in humans. One study included 4 male and 7 female vervet monkeys fed cultured material containing FB_1, FB_2 and FB_3 (FINCHAM et al., 1992). Animals were feeding for 40-573 days with fumonisins in a range of from 784 to 3257 mg/vervet/day, and from 310 to 748 days with fumonisins in a range of from 392 to 814 mg/vervet/day. The results showed cholesterolemia, raised plasma fibrinogen and activity of Factor VII, which enhance atherosclerotic response. In addition, chronic hepatotoxicity was observed, which is in agreement with findings in other animals, although no carcinogenic activity was observed.

SHEPARD et al. (1996) investigated the disruption of the sphingolipid metabolism in vervet monkeys. Animals were consuming diets containing *F. moniliforme* culture material, with an approximate daily intake of 0. 3 and 0.8 mg/kg body mass/day. Although no significant differences were found in serum levels of sphingosine compared to controls, serum sphinganine levels in the experimental groups were significantly elevated from a mean of 0.43 in controls, and 1.72 and 2.57 in experimental groups. Similar changes were established in urine with an increase of in the ratio from 0.87 in controls to 1.58 and 2.17 in experimental groups. Therefore, elevation of Sa/So ratio, as consequence of disrupted sphingolipid metabolism by fumonisisn, can be monitored in the serum of exposed animals.

Culture material of *F. moniliforme* strain MRC 826 was fed to male and female vervet monkeys for 13.5 years (GELDERBLOM et al., 2001a; ANONYMOUS, 2001). Blood chemical analyses were conducted bimonthly; all clinical signs were recorded and liver biopsy samples were taken at intervals up to 4.5 years. The threshold dose of fumonisin B for kidney and liver damage was estimated to be between 0.11 and 0.18 mg FB/kg body mass (bm) (equivalent to 8.21-13.25 mg/kg of diet). Cholesterol and creatine kinase were also adversely affected. White and red blood cells were significantly decreased in the treated animals, while the serum sphinganine level and the sphingosine/sphinganine ratio were significantly increased. The lowest observable effect level (LOEL) for sphingolipid changes in the serum was 0.29-0.64 mg/kg bm per day (equivalent 22-48 mg/kg of diet).

Prevention and detoxification of fumonisins

The prevention of contamination of corn with fumonisins may be achieved by the use of resistant corn varieties, insect control and seed treatment (MARASAS, 1995). The use of physical or chemical methods may accomplish detoxification. The effect of heat on fumonisin aqueous solution to 150 ^{0}C or higher, or heating moist corn kernels, indicate a significant reduction of fumonisin concentration (JACKSON et al., 1996). However, heating causes hydrolysis of the primary amine group of the fumonisins, leaving the backbone of the molecule intact. Toxicity of the hydrolysed products has been demonstrated in some experimental systems. Chemical detoxification of fumonisin-contaminated corn can be achieved by treating it with $Ca(OH)_2$, and significant reductions of FB_1 (up to 95%) have been reported (MUNKVOLD and DESJARDINS, 1997). However, there are reports on the toxicity of hydrolysed end products, which found that hydrolysed fumonisin may be as toxic as unaltered FB_1. Ammoniation may successfully detoxify the fumonisins when combined with high temperature, while low temperature ammoniation has been unsuccessful (PARK et al., 1992; SYDENHAM et al., 1995). A promising method for detoxification was recently reported. Non-enzymating browning is a reaction that occurs in the presence of a primary amine, a reducing sugar, and water pH>7. This reaction results

in the removal of the primary amine group from the fumonisin. Treatment of FB_1 with fructose under these conditions resulted in a significant reduction in detectable FB_1. When a diet containing the products of the FB_1-fructose reaction was fed to rats, it was non-toxic and did not result in cancer initiation (LU et al., 1997). PARK et al. (1996) reported that treatment of corn with H_2O_2 and $NaHCO_3$ reduced fumonisin concentrations by up to 100%. Toxicity of the end product was greatly reduced compared with untreated corn. Recently, CASTELO et al. (2001) reported 92.1% loss of FB_1 when corn –based foods were extruded with glucose.

Carcinogenic risk assessment and control

In accordance with the findings of many researches carried out on animals, the International Agency for Research on Cancer (IARC; 1993) classified fumonisins B_1 and B_2 as 2B carcinogen, which means that sufficient evidence exists of the carcinogenic effect on animals, while there is insufficient evidence of these effects in humans (CASTEGNARO and

Table 1. Recommended levels for total fumonisins ($FB_1 + FB_2 + FB_3$) in feed

Animals	I	II	III
Equids and rabbits	5	0,2	1
Rabbit	5	0,2	1
Catfish	20	0,5	10
Swine	20	0,5	10
Ruminants (cattle, sheep, goats and other ruminants that are ≥ 3 months old and fed slaughter)	60	0,5	30
Mink (fed for pelt production)	60	0,5	30
Poultry (turkeys, chickens, ducklings and other fed for slaughter)	100	0,5	50
Ruminant, poultry and mink breeding stock (includes laying hens, roosters, lactating dairy cows and bulls)	30	0,5	15
All others (includes dogs and cats)	10	0,5	5

I = recommended maximum levels of total fumonisins ($FB_1 + FB_2 + FB_3$) in corn and corn by-products (ppm)

II = feed factor (fraction of corn or corn by-product mixed into total ration)

III = recommended maximum levels of total fumonisins ($FB_1 + FB_2 + FB_3$) in the total ration (ppm)

MCGREGOR, 1998). The worldwide contamination of corn with fumonisins makes it advisable to determine reasonable tolerance levels in corn and corn products for human and animal nutrition. The Mycotoxin Committee of the American Association of Veterinary Laboratory Diagnosticians recommended FB_1 limits in feed as: < 5 ppm Equidae, < 10 ppm swine, < 50 ppm poultry and beef cattle (RILEY et al., 1993b). The U.S. Food and Drug Administration (http://www.cfsan.fda.gov., 2001) recommended levels for total fumonisins in feed (Table 1), and in human foods.

Conclusions

Many studies have been carried out on fumonisins in the past decade in order to establish their effects on animals. These studies cannot be described as preliminary, but many questions still remain unanswered.

Since fumonisins occurs in a high number of stereoisomers, occurrence in corn, and the possible toxic effects of iso-FB_1, need to be investigated.

Considering chemical structure and polarity, how are fumonisins transported through the membrane system to exert their action in affected cells?

Fumonisin B_1 induces cancer in rodents.

Fumonisins exert a toxic effect on all animals treated, with considerable differences in the response of various species. Why?

Since fumonisins produced renal toxicity in most animal species, including swine, do they have any possible role in the etiology of endemic nephropathy?

Fumonisin B_1, as natural toxicant, can be used for further characterisation of the sphingolipid metabolism, regulation of apoptotic pathways, and renal injury.

Fumonisins produced no oesophageal cancer in any animal species tested, including primates. Potential role of fumonisin carcinogenesis in humans is yet to be established.

Since the Sa/So ratio was found to be an adequate biomarker of early exposure to fumonisins, it is necessary to apply these examinations on animals, especially in regions of high incidence of fumonisins in corn.

Control of fumonisins to the levels recommended by the U. S. FDA can reduce the risk for human and animal health.

A great deal of work is needed to develop an efficient detoxification method that can be used in a commercial setting.

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SAŽETAK

Fumonizini su mikotoksini koje sintetiziraju *Fusarium* vrste, poglavito *Fusarium moniliforme*, svjetski rasprostranjen zagađivač kukuruza. Poznato je da se fumonizini dovode u vezu s različitim animalnim toksikozama i karcinomima, uključujući leukoencefalomalaciju (ELEM), plućni edem u svinja (PPE), hepatotoksikozu, hepatokarcinome, nefrotoksikozu i imunosupresivni učinak. Među fumonizinskim izomerima, FB_1 je predominantan molekularni oblik i glavni toksični metabolit. Utvrđeno je da fumonizini ometaju metabolizam sfingolipida u različitim stanicama i tkivima inhibicijom ceramid-sintaze, ključnog enzima koji sudjeluje u *de novo* biosintezi sfingolipida i reacilaciji slobodnog sfingozina. Drugi mehanizam toksičnosti FB_1 uključuje promjene polinezasićenih masnih kiselina i membranoznih fosfolipida. Različiti toksični učinci FB_1 bilježeni su u mnogih vrsta životinja, primjerice ekvida, svinja, štakora, miševa, zečeva, peradi, primata i drugih. Prijeko je potrebno provesti procjenu izloženosti, primijeniti legislativu, te dostatne detoksifikacijske metode kako bi se izbjegle neželjene posljedice i spriječili ekonomski gubitci.

Ključne riječi: fumonizini, *Fusarium moniliforme*, animalne toksikoze, ceramid-sintaza, fosfolipidi