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Invited Review

In vivo toxicity studies of fusarium mycotoxins in the last decade: A review

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ABSTRACT

This review summarizes the information regarding the *in vivo* studies of *Fusarium* mycotoxins in the last decade. The most common studies are classified as subacute toxicity, subchronic toxicity, acute toxicity, toxicokinetic studies and teratogenicity in order of importance. The most used animals in *in vivo* studies are pigs, rats, chickens and mice. Fumonisin B1, deoxynivalenol, zearalenone, nivalenol and T-2 toxin are the most studied fusarotoxins. Studies with combinations of mycotoxins are also frequent, deoxynivalenol generally being one of them. The predominant route of administration is oral, administered mostly in the form of naturally contaminated feed. Other administration routes also used are intraperitoneal, intravenous and subcutaneous. *In vivo* research on *Fusarium* mycotoxins has increased since 2010 highlighting the need for such studies in the field of food and feed safety.

1. Introduction

1.1. Fusarium *genera*

The genus *Fusarium* is a large fungal form genus that is more than hundreds of years old, and globally one of the most important genera of fungi. Its species, which invade agriculturally important grains, are probably the most prevalent toxin-producing fungi of the northern temperate regions and are commonly found in cereals grown in America, Europe and Asia (Tiemann et al., 2006). Most members of the genus produce – under favorable environmental conditions – an array of secondary metabolites, which vary widely in chemical form. A number of the secondary metabolites are important as mycotoxins that are toxic and/or carcinogenic to humans and animals

Abbreviations: ADME, absorption, distribution, metabolism and excretion; AFs, aflatoxins; ALT, alanine aminotransferase; HFB1, aminopentol; AS, aluminosilicate; AST, aspartate aminotransferase; AUC, area under curve; BEA, beauvericin; bw, body weight; CAT, catalase; CYP, cytochrome P-450; D3G, deoxynivalenol-3-β-D-glucoside; DES, diethylstilbestrol; DNA, deoxyribonucleic acid; DOM-1, deepoxy-deoxynivalenol; DON, deoxynivalenol; DONS, deoxynivalenol sulfonate; DON-3-GlcA, deoxynivalenol-3-glucuronide; DON-15-GlcA, deoxynivalenol-15-GlcA; EFSA, European Food Safety Authority; ENNs, enniatins; ENN A, enniatin A; ENN B, enniatin B; EPT, 12,13 epoxytrichothec-9-ene; EU, European Union; FAO, Food and Agricultural Organization of the United Nations; FBs, fumonisins B; FB_1, fumonisin B_1; FUS, fusaproliferin; GMA, glucomannan mycotoxin binder; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; HDL, high density lipoprotein; HT2, HT-2 toxin; HT29, human tissue colon cell line; IARC, International Agency for Research on Cancer; IP, intraperitoneal; IV, intravenous; LD50, lethal dose 50%; LDL, light density lipoprotein; LOQ, limit of quantification; LPS, lipopolysaccharides; MAPKs, mitogen-activated protein kinases; MDA, malondialdehyde; MON, moniliformin; mRNA, messenger ribonucleic acid; NIV, nivalenol; NOAEL, no-observed-adverse-effect level; PMTDI, provisional maximum tolerable daily intake; OECD, Organisation for Economic Co-operation and Development; PBMC, peripheral blood mononuclear cells; OTA, ochratoxin A; PBMC, peripheral blood mononuclear cells; PHFB1, partially hydrolyzed fumonisin B1; PUBMED, Publications of Medicine; Sa, sphinganine; Sa 1-P, sphinganine 1-phosphate; SBS, sodium metabisulfite; SC, subcutaneous; Se-GSH-Px, selenium dependent glutathione peroxidase; So, sphingosine; So 1-P, sphingosine 1-phosphate; SOD, superoxide dismutase; T2, T2 toxin; TNF, tumor necrosis factor; WHO, World Health Organization; WOS, Web of Science; ZEA, zearalenone; ZOL, zearalenol.

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and may have a role in plant disease. These mycotoxins are commonly found in cereal food and feed and in other animal products consumed daily. They should be regulated in commercial and international trade. Mycotoxins possess biological activities that have been shown in many different studies and may represent a problem to both human and animal health.

The diseases that *Fusarium* species cause, the toxins they produce and the general social impact on agricultural communities are an ongoing problem (Summerell and Leslie, 2011). *Fusarium* mycotoxins are endowed with both acute and chronic toxic effects and have been shown to cause a broad variety of toxic effects in animals. The consequences of ingestion of these fungal compounds vary from acute, overt diseases with high morbidity and death to chronic disease, decreased resistance to pathogens and reduced animal productivity. However, the major problem associated with animal feed contaminated with mycotoxins is not acute disease episodes, but rather the ingestion of low levels of toxins which may cause an array of metabolic, physiologic, and immunologic disturbances. Symptoms related to mycotoxicosis can occur at very low toxin concentrations, even below the detection limits for the current analysis methods, and clinical symptoms are in many cases not very pronounced (Kanora and Maes, 2009). In addition, as it is a common practice to use multiple grain sources in animal diets, the risk of exposure to several mycotoxins increases with diet complexity (Grenier and Applegate, 2013).

Knowledge of the effects of mycotoxins is expanding rapidly, mainly because of the development of novel analytical techniques that facilitate the study of these compounds (Kanora and Maes, 2009). Mycotoxin research into effects on intestinal functions has made substantial progress in recent years. The intestinal epithelium is the major site for the effects of mycotoxin-contaminated material, even at low levels of contamination. The intestinal tract is the first barrier against ingested antigens, including mycotoxins and pathogenic bacteria. Following ingestion of mycotoxin-contaminated food, enterocytes may be exposed to high concentrations of toxins. A role of food-associated mycotoxins in the induction or persistence of human chronic intestinal inflammatory diseases has also been suspected. Studies focusing on the influence of food-derived antigens on intestinal morphology as an indicator of animal health are common but there are few publications on the effects of chronic exposure to a mycotoxin co-contaminated diet (Loureiro-Bracarense et al., 2012). Studies on the effect of these compounds on the gastrointestinal tract are limited. Studying the occurrence of any given mycotoxin alone provides incomplete information about the risk associated with the respective feedstuff. Compound feed is particularly vulnerable to multiple contaminations as it typically contains a mixture of several raw materials. *Fusarium* mycotoxins in general are often found to occur together in contaminated cereals. In most cases, the resulting toxic effects will be additive combinations of the mycotoxins' individual toxicities but synergistic interactions have been observed (Streit et al., 2012).

Spontaneous outbreaks of *Fusarium* mycotoxicosis have been reported in Europe, Asia, Africa, New Zealand, and South America. Moreover, chronic intake of these mycotoxins is reported on a regular and more widespread basis due to their global occurrence (Cortinovis et al., 2013).

1.2. Fusarium *mycotoxins*

From the large variety of known mycotoxins, the major *Fusarium* mycotoxins are, besides aflatoxins (AFs), the most prevalent and harmful mycotoxins to animal productivity and responsible for extensive and recurring economic damage. These toxins inflict loss to farmers and reduce the value of contaminated feeds (Grenier and Applegate, 2013). *Fusarium* species produce three of the most important classes of mycotoxins with respect to animal health and production: fumonisins (FBs), zearalenone (ZEA), and trichothecenes (deoxynivalenol (DON), nivalenol (NIV), T-2 and HT-2 toxins), but *Fusarium* genera also produce emerging mycotoxins, such as fusaproliferin (FUS), beauvericin (BEA), enniatins (ENNs) and moniliformin (MON), which are more recently discovered and less studied (Summerell and Leslie, 2011).

1.2.1. Trichothecenes

Trichothecenes are the main and the chemically most diverse chemical group of the three major classes of *Fusarium* mycotoxins (Summerell and Leslie, 2011). They represent a large family of chemically related toxins produced by fungi in taxonomically unrelated genera, such as *Fusarium*, *Myrothecium*, and *Stachybotrys* and present a potential threat to animal health throughout the world (Li et al., 2010). The broad family of trichothecenes is extremely prevalent and their molecular weights range between 200 and 500 Da. The trichothecenes family includes over 200 toxins with a sesquiterpenoid structure with or without a macrocyclic ester or an ester–ether bridge between C-4 and C-15. They contain a common 12,13-epoxytrichothecene group responsible for their cytotoxicity and a 9,10 double bond with various side chain substitutions. They have been classified into four groups (Types A, B, C, and D) based on the substitution pattern of the tricyclic 12, 13 epoxytrichothec-9-ene (EPT) core structure. Types A, B and C can be differentiated based on the substitution at the C-8 position. The non-macrocyclic trichothecenes constitute two groups: Type A that has a hydroxyl, ester or non-oxygen substitution group at C-8, including T-2 toxin, HT-2 toxin, neosolaniol and diacetoxyscirpenol, while the Type B group contains a keto (carbonyl) function at C-8 and includes fusarenon-X, nivalenol (NIV) and deoxynivalenol (DON) and its 3-acetyl and 15-acetyl derivatives. In *Fusarium*, Type B trichothecenes typically have a C-7 hydroxyl group, but this structural feature is not present in other genera. The number and position of the hydroxyl and acetyl-ester groups can influence the relative toxicity within eukaryotic cells. Type C trichothecenes have a C-7/C-8 epoxide (crotocin) and type D trichothecenes have an additional ring linking the C-4 and C-15 positions (roridin A, verrucarin A, satratoxin H) (McCormick et al., 2011; Pinton and Oswald, 2014).

Trichothecenes are small, amphipathic molecules that can move passively across cell membranes. They are easily absorbed via the integumentary and gastrointestinal systems, allowing for a rapid effect of ingested trichothecenes on rapidly proliferating tissues (Pinton and Oswald, 2014). Trichothecenes are toxic to animals and its exposure has been linked to reproductive disorders in domestic animals (Cortinovis et al., 2013). Because of their effects on the immune system, the exposure of trichothecenes could predispose humans and animals to infectious disease, especially for sensitive human populations like young children, immuno-depressed people and old people (Gouze et al., 2007).

Exposure to these toxins can cause feed refusal, immunological problems, vomiting, skin dermatitis, and hemorrhagic lesions. They are also phytotoxic and can cause chlorosis, inhibition of root elongation, and dwarfism, and act as a virulence factor in wheat head scab (McCormick et al., 2011). The adverse effects of trichothecenes include emesis, nausea, anorexia, growth retardation, neuroendocrine changes and immunosuppression. In humans, there is a body of evidence suggesting that trichothecenes cause acute illness and are frequently associated with outbreaks of gastroenteritis. At the molecular level, trichothecenes display multiple inhibitory effects on the primary metabolism of eukaryotic cells including the inhibition of proteins, DNA and RNA synthesis. This impairment leads to the alteration in cell proliferation in tissue with high rates of cell turnover such as intestinal epithelial cells. Thus intestinal epithelial cells are especially sensitive to trichothecenes and their exposure to these toxins may induce toxicity (Alassane-Kpembi et al., 2013). Exposure to DON and other *Fusarium* mycotoxins generally

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