Toxicology of Deoxynivalenol

Subjects: Agriculture, Dairy & Animal Science
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Deoxynivalenol is a toxic compound produced by filamentous fungi and represents a threat to public health. It is not possible to totally extinguish fungal contamination in crops such as wheat and corn and thereby avoid the production of this toxin.

Keywords: Deoxynivalenol ; Phylogeny ; DON ; Mycotoxins

1. Introduction

Mycotoxins are toxic secondary metabolites produced by fungi that infect crops and can be produced in the field, during postharvest procedures, and in storage. The main genera involved in mycotoxin production are *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, and *Penicillium* [1]. Around 400 mycotoxins have been described so far, and they differ in their structure, metabolization, and level of toxicological effects [2]. However, they are mostly stable to thermal processes and have negative effects on human and animal health [3].

Up to 80% of the grains produced worldwide are contaminated with at least one mycotoxin, but cooccurrence of two or more mycotoxins is very common, increasing their risk to human and animal health [4].

Among the mycotoxins characterized, trichothecenes are a group of sesquiterpenoids produced by *Fusarium* sp. that comprises deoxynivalenol (DON) and its acetylated forms, nivalenol (NIV), T-2 toxin, and HT-2 toxin [5]. In recent years, research on DON's toxicology and mitigation methods has become more common because of DON's high incidence across the world.

2. Occurrence, Regulation, Ingestion, and Metabolism

Grain contamination by mycotoxins may occur in the field or during storage, and factors such as temperature, high humidity, and handling are key points, as they can favor the production of mycotoxins [6].

DON is commonly found in temperate areas. Studies have reported that no DON has been identified in grains kept at water activity (a_w) < 0.9 and temperatures lower than 11 °C [7]. Hope et al. [7] demonstrated that for both *Fusarium graminearum* and *F. culmorum*—main DON producers—ideal conditions for the toxin's production are 25 °C and a_w > 0.98, which is in agreement with Ramirez and colleagues [8]. The use of fungicides can also stimulate DON's production, especially in low doses, as ineffective doses promote mild to medium levels of stress to the fungus [7]. Among the practices of crop handling—e.g., fungicide and fertilizer application—the use of *Fusarium*-resistant cultivars seems to have the greatest positive influence on maintaining low levels of DON in grains. These cultivars were developed to present different genes that promote resistance to *Fusarium* head blight, a common wheat disease associated with DON [9].

DON, also known as vomitoxin, is the most prevalent mycotoxin according to the last survey report from Biomin [10], a referenced company in the field of mycotoxins, which analyzed 21,709 maize and wheat samples from all continents of the world. The study indicated high prevalence of DON in China (mainland and Taiwan), Middle East and Central America, where 86%, 78%, and 76% of the samples, respectively, were contaminated with the toxin. In South America, wheat samples presented an average of ≥1.5 µg/g of DON, representing a high risk for animal production, especially swine. In Oceania, this toxin was found in only 18% of samples. It is noteworthy that Oceania presented the lowest rate of all mycotoxins analyzed in this survey, and the risk for animal production was considered low to moderate. Higher concentrations of DON were observed in the 2020 survey than in the survey performed by Biomin in 2019, increasing the demand for more effective solutions for this issue.

Considering the panorama presented, regulations were established by governments enforcing maximum tolerable levels (MTL) of the toxin in foods and animal feed. Food intended for humans has its own regulation, which is more severe than that for animal feed, especially for foods destined for infants, who are more susceptible to the toxic effects of DON [11].

In 2004, the Food and Agriculture Organization (FAO) [12] released a worldwide survey on mycotoxin regulation status comparing the situations in 2003 and 1995. DON regulations were found in more countries after these nine years, although many of them considered these limits only for foods and not feed. In 2016, Romer Labs, a renowned company in the mycotoxin field, also released a survey showing that some countries are now regulating DON's presence in feed [13]. Taken together, these data show a tendency toward more severe regulations, expanding DON control to other crops besides maize and wheat.

Legislation varies widely among the regulatory agencies of different countries. The European Commission (EC) has established detailed legislation, applying lower limits to swine production (0.9 µg/mL) than the Food and Drug Administration (FDA) of the United States (FDA), which recommends 5 µg/mL. South Africa and Canada established similar levels to those of the EC (1 µg/mL). **Table 1** summarizes legislation established by the FDA [14]; the Canadian Food Inspection Agency [15]; the South African Department of Agriculture, Forestry, and Fisheries [16]; and the European Commission [17].

Table 1. Recommended levels of deoxynivalenol in animal feed established by regulatory public agencies worldwide.

Agency	Specifications	Limit (mg/kg)	Reference
FDA (United States)	Grains and grain by-products destined for ruminating beef and feedlot cattle older than 4 months and for chicken	10	[14]
	Grain and grain by-products destined for swine and other animals	5	
EFSA (EU)	Cereals and cereal products except for maize by-products	8	[17]
	Maize by-products	12	
	Complementary and complete feeding stuffs for animals	5	
	Complementary and complete feeding stuffs for pigs	0.9	
Food Inspection Agency (Canada)	Diets for cattle and poultry	5	[15]
	Diets for swine, young calves, and lactating dairy animals	1	

Agency	Specifications	Limit (mg/kg)	Reference
Department of Agriculture, Forestry, and Fisheries (South Africa)	Feeding stuffs on a full ration basis for:		[16]
	Pigs	1	
	Cattle	5	
	Calves up to 4 months	2	
	Dairy Cattle	3	
	Poultry	4	

Some countries do not have official guidelines on MTL for animal feed. In Asia, each country makes its own regulations, and according to the Romer Labs report, China and Japan are the only countries that recommend MTL for DON in animal feed. Japan covers only specific groups, such as cows over 3 months of age [13]. Australia does not present specific regulations for this toxin in feed, probably because of the low levels found in the country and, therefore, the low incidence of mycotoxicosis in animals [18]. It is expected that in the coming years, with increased visibility of DON's effects on the food chain supported by consistent research, new regulations will be established.

Deoxynivalenol MTLs were set only on cereals because DON presents a low occurrence in other products. Cereals are the main entry point for this mycotoxin in daily food and feed, because grains are a staple worldwide, especially maize and wheat. Swine and broilers are heavily exposed to DON, as their diets are composed mainly by grains, compared to other production animals [19].

Toxin daily intake is hard to measure in animals because of the different nutrient requirements of each species and even during different growth phases within the same species. Furthermore, as many countries do not have specific legislation for animal feed, and several types of feed can be used in this process, it is even harder to obtain a general estimate of the situation. What is certain is that both humans and animals are constantly exposed to DON, as it is not possible to totally extinguish fungal contamination in crops such as wheat and corn and thereby avoid the production of this toxin.

Once it is ingested by an animal, DON metabolization occurs in the intestine. In this process, some metabolites such as DON-3S, DON-GlcA, and DOM-1 may be generated in broilers [20] and in swine. The high transformation of DON into DON-3S and rapid elimination of the parent toxin may be the reasons why poultry is less susceptible to the effects of the toxin [20]. A suggested route for DON metabolism in poultry is its absorption in jejunum, transformation to DON sulfate forms (DON-3-S and DON-15-S) in the intestine, and excretion through bile and urine [21].

Swine are more sensitive to DON because of their high rate of absorption of the toxin in the upper digestive system, especially in the small intestine. Urine was found as the main excretion route, with DON being the best biomarker in this matrix, indicating a lower rate of metabolization by pigs than by poultry [22]. Microbiota composition is also a key factor for this toxin's metabolization, as some microorganisms are capable of transforming the toxin into less toxic compounds, although this is not common in swine [23]. Fast tissue distribution was also observed, with 98% of metabolization occurring after 12 to 24 h; only traces were identified after this period [24]. **Figure 1** summarizes poultry's and swine's main DON metabolization routes by oral ingestion.

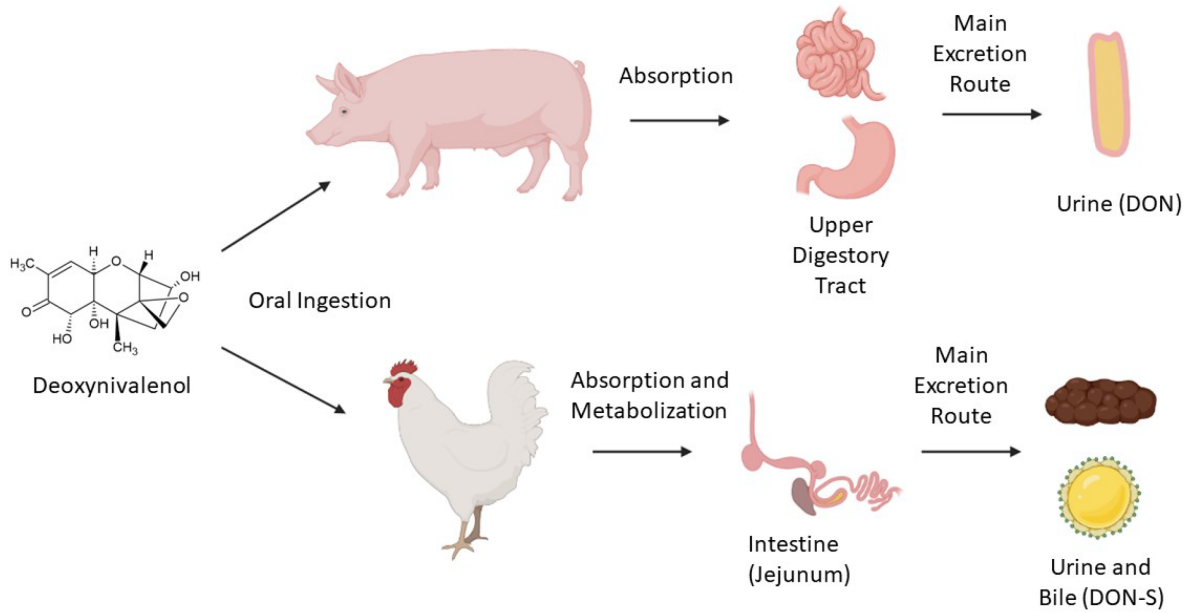

Figure 1. Main metabolization routes (simplified) of deoxynivalenol ingested orally by swine and poultry.

Lack of research and adequate regulations for mycotoxins are a risk to animal feed safety worldwide. Regarding DON, most research has focused on swine, followed by poultry, and the metabolism in these organisms is well documented. In ruminants, recent studies demonstrating the metabolism of vomitoxin are scarce. Ruminants are considered resistant because of their robust microbiota, which can transform the toxin into less toxic metabolites [25]. However, evidence has pointed to modulatory effects of mycotoxins on the intestinal microbiota of ruminants, which must be further investigated [26].

Soon, more severe control of DON is expected, as MTLs for this toxin tend to be included in countries where it is not regulated. It should also be more severely controlled than the existent regulations, especially as climate change can favor its production [27].

3. DON's Mechanisms of Toxicity

DON is classified as a sesquiterpenoid and possesses in its structure an epoxy group at C12–13 and hydroxyl groups at C3, C7, and C15, which are mainly responsible for its toxicity [28][29].

Recent studies have shown a change in gene regulation as one effect of DON exposure, mainly affecting immune response genes, especially those linked to cytokines, which are signaling molecules that regulate the inflammatory response. There has also been evidence of disruption in the expression of genes related to nutrient transport, barrier function, cell cycle regulation, and mitochondrial function, leading to malfunction of the animal cell [30][31]. In high doses (e.g., 8 µg/g of feed), DON can suppress genes related to immune response [32]. DON has also presented upregulation of apoptotic gene expression, leading to cell death of hippocampal nerve cells in piglets [33].

At a molecular level, DON affects ribosomal activity by binding into the 60S unit and inducing ribotoxic stress, leading to deficient protein synthesis. Changes in the mitochondrial structure and functioning were also observed. It also causes activation of mitogen-activated protein kinases (MAPK), leading to impairment of cell proliferation and apoptosis [33].

DON exerts its toxicity mainly in the gastrointestinal tract (GIT), and when in high doses, it provokes a reduction in goblet cell production. These cells are responsible for mucus production and help to maintain the integrity of the intestinal barrier. DON also affects the expression of tight junction proteins, such as claudins, that are responsible for regulating epithelial cell permeability and cell adhesion in the intestine [34]. This is especially worrying because the GIT enables adequate nutrient absorption, and this function may be impaired.

The intestinal barrier and a healthy microbiome also protect the animal against pathogens, and they are both negatively affected by DON [35]. Differences between the microbiota in the small intestines of weaning piglets fed with DON and those fed with a DON-free diet were reported [36]. Clear signals of dysbiosis, such as decrease in the population of Firmicutes—involved in the metabolism of nutrients and maintenance of intestinal health—and increased presence of Actinobacteria were noticed in piglets fed with DON. Similar results were found in weaning rabbits, with decreased microbiota diversity under the presence of high levels of DON [37].

Microbiota also play an important role in protecting the host from pathogen growth along with the immune system, which also suffers under the effects of DON. Changes in the T-cell differentiation pattern, decreasing the proliferation of cells that are directly involved in immune response, were found [38]. This result was supported by Cai and colleagues [39], who described a decrease in naïve cell differentiation into antibody-secreting cells due to lower cytokine receptor expression on the cell surface. Furthermore, they demonstrated that the toxin affected the immune response of mice infected with *Listeria monocytogenes*, intensifying the infection.

Alterations in the reproductive cycle have also been noticed in animals intoxicated with DON. The mycotoxin (2 µg/mL) has provoked disruption in the hormonal cycle, stimulating the release of progesterone and estrogens in vitro in porcine ovarian granulosa cells [40]. Disruption of the histological pattern and impairment of follicular development in ovarian explants of pigs were also demonstrated [41].

The toxin also restrained testicular development causing anomalies in its structure and impaired blood–testis barrier integrity in mice [42]. Sperm viability was also decreased, and morphology alteration of the gametes was found, results that were supported by Tassis et al. [43] in their study with boar semen and Yang et al. [44] in their study with BALB/c mice. One study also indicated that testicular function was not the only factor negatively affecting the male reproductive function and that neuroendocrine activity may suffer important alterations as an effect of DON [42]. Altered activity in the brains of piglets was suggested, especially in the release of neurotransmitters responsible for physiological and nervous system regulations, such as decreases in dopamine and GABA and increases in norepinephrine and 5-hydroxytryptamine [45]. One possible effect is the modulation of appetite.

The brain cell morphology of piglets was also altered by DON, with a lack of organelle and vacuole formation when challenged with 2.2 µg/g of the toxin added to the feed. DON also decreased the antioxidant activity in the brain because of a reduction in superoxide dismutase and glutathione peroxidase activity [45]. Furthermore, an increase in blood–brain barrier permeability and a decrease in cell viability were found in in vitro models as well as in rats, chickens, pigs, and mice. All together, these studies point to brain activity disorders and homeostasis imbalance [46].

Other studies have reported that other organs, namely the liver [47], kidney, and spleen [48], are also affected by DON. This results in immunosuppression, metabolic alterations, and disturbance in the amino acid production profile, leading to malfunction of physiological processes. **Figure 2** summarizes the effects of DON on targeted animal organs and systems.

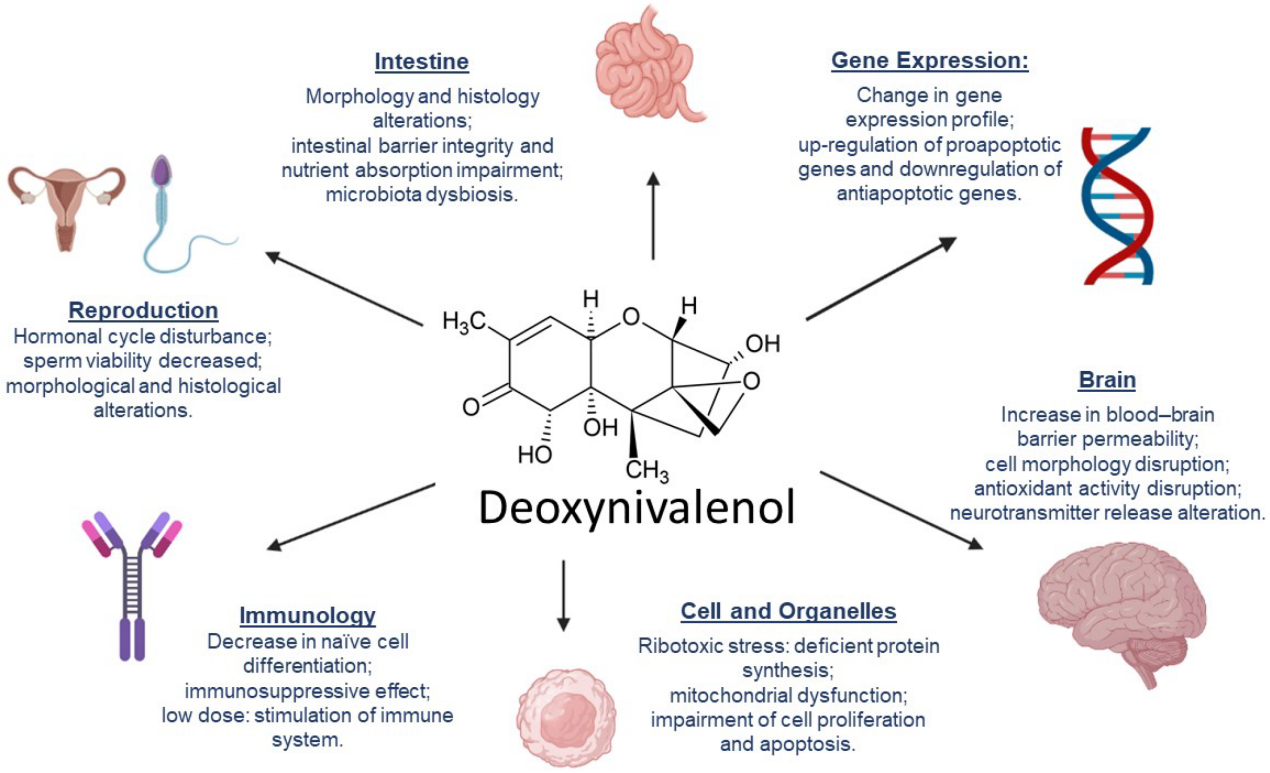

Figure 2. Effects of deoxynivalenol in animal organs and systems.

DON's toxicity in vivo is well documented. Negative impacts were reported in grass carp [49], broilers [50][51], piglets [36][52], finishing pigs [53], mice [54][55], and rabbits [56]. Among food animals, a predominance of studies involved pigs and piglets rather than broilers, probably because of the higher tolerance to the toxin presented by broilers. Important factors to be considered for the severity of the toxin's effect are dose and time of exposure, which determine the outcome of the

intoxication. Serviento and colleagues [53] compared three treatment groups of finishing pigs fed with 3 µg/g of DON. The first group was exposed to the toxin once a day from 113 to 119 d of life; the second group was exposed to DON once a day from 134 to 140 d; and the third group was exposed to the toxin in both periods. The results suggested that pigs' tolerance to DON's presence increased in the second exposure after 4 weeks, probably because of adaptations of their microbiota. They also showed that previous contact with the toxin did not avoid adverse effects of later exposures. However, it improved animal recovery from a second exposure to contaminated feed. In addition, it was observed that older animals exposed to the toxin presented lower average daily feed intake and daily weight gain than those challenged in early periods, indicating that age is also an important parameter to be considered.

Studies have shown immunosuppression in animals subjected to high doses of the toxin [29][37][38][56]. However, a recent study showed that low doses of DON can stimulate the immune system, increasing lymphocyte and goblet cell numbers and activating signaling pathways with an increased production of cytokines, suggesting a dose-dependent effect in the immune response [57]. Alassane-Kpembi et al. [58] pointed out that commonly used detection methods may fail to identify the potential harms from low-exposure doses of DON and that omics have the potential to provide specific fingerprints about the mycotoxin's effects.

The masked forms of DON also represent a threat to food security, and they are often neglected. Acetyl and glycosylated modifications are among the most common masked forms, and some of them may be more toxic than DON itself. Studies have shown faster absorption of acetylated forms and toxic effects, such as activation of the MAPK signaling pathway, similar to those of DON. In addition, digestive enzymes and microorganisms can transform 15-A-DON and 3-A-DON in DON [59].

Because of the diverse toxic effects of the toxin in different systems of the animal organism, some strategies have been developed to mitigate them. Biodegradation is a potential mechanism to decrease DON's toxicity and has been widely studied in recent years. The most reported biodegradation metabolites are DOM-1 and 3-epi-DON, which were found to be less toxic than the parent toxin [60][61]. Their characteristic lower toxicity was confirmed by Bracarense and colleagues [52] in vivo using piglets fed with 3 µg/g of DON, DOM-1, and 3-epi-DON. Results demonstrated histological modifications and proinflammatory response in the intestines, livers, and lymph nodes of animals treated with DON. However, those treated with DOM-1 and 3-epi-DON presented similar scores as those in the control group.

Reduction in T-cell proliferation and disruption to the expression of molecules involved in immune response were observed in vitro when cells were exposed to 1.6 µM of DON, yet a 10-fold higher dose of DOM-1 did not exert any of these effects [62]. Mayer and colleagues [63], working with five different cell lines—mice macrophages (RAW 264.7), porcine intestinal cells (IPEC-1 and IPEC-J2), trout gill (RTgill-W1), and human liver cell (HepG2)—found similar results.

Toxic effects of DON are well documented, especially in swine, which has been found the most sensitive species to DON in animal production. Although many in vitro studies have reported a sharp drop in toxicity via microbial transformation, generating DOM-1 and 3-epi-DON, there is a lack of studies of in vivo toxicity, especially about 3-epi-DON, which was described in the literature later than DOM-1.

In vivo analyses often require special evaluation and authorization from the ethics committee on the use of animals, an additional step that is often bureaucratic and time consuming, although necessary. Further in vivo studies are required to fully confirm the lower toxicity of these metabolites, as systemic effects cannot be fully evaluated in in vitro studies.

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