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Dietary exposure to the food contaminant deoxynivalenol triggers colonic breakdown by activating the mitochondrial and the death receptor pathways.

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Abstract

The food contamination by mycotoxins is of increasing public health concerns. Deoxynivalenol (DON), a mycotoxin contaminating cereals has been associated with the exacerbation of inflammatory bowel diseases (IBD), thereby raising the question of its role in the development of IBD. The effect of DON on the colon is poorly described. Exposure of Wistar rats to low doses of DON (2 or 9 mg.kg⁻¹ feed) for one to four weeks induced microscopic alterations of colonic tissue which were characterized by dilated lymphatic vessels, luminal necrotic debris, and cubic and flattened enterocytes. Ingestion of DON also altered colonic functions by increasing paracellular permeability while reducing the expression of the tight junction proteins. Exposure to DON also increased apoptosis in colonic tissue through both the mitochondrial (intrinsic) and the extrinsic pathways: increase of the protein expression Bax/Bak, cytochrome C and caspase 9 whereas expression of anti-apoptotic protein Bcl2 tended to decrease (mitochondrial pathway); increased expression of FasR and caspase-8 and no change of the expression of the protein adaptor FADD (extrinsic pathway). An increase in the pro-inflammatory markers TNF α , IL-17 and myeloperoxidase was also observed. Taken together, these results indicate that the dietary exposure to low levels of DON targets the colon inducing a health-threatening breakdown of the colonic barrier, highlighting oral exposure to DON as a potential risk factor in triggering IBD.

Abbreviations

DON = deoxynivalenol
EFSA = European Food Safety Authority
JEFCA = Joint FAO/WHO Expert Committee on Food Additives
TDI = Tolerable Daily Intake
MPO = myeloperoxidase
U = units
IBD = inflammatory bowel disease
R = electrical resistance
FSS = Fluorescein Sodium Salt
HRP = Horseradish peroxidase
TUNEL = transferase-mediated tetramethylrhodamine-dUTP (TMR-dUTP) nick end labeling
FasR = Fatty acid synthetase receptor
FADD = Fas-associated death domain
IL-x = Interleukin – x
CXCL1 = chemokine (C-X-C motif) ligand 1
TNF α = tumor necrosis factor α
GIT = Gastro-Intestinal tract
ZO-1 = Zonula Occludens
TJ = Tight Junction
NOAEL = no observed adverse effect level
JNK= JunN-terminal kinase
NFkB = nuclear factor kB

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1. Introduction

Mycotoxins are the most frequently occurring natural food contaminants (Payros et al. 2021). Among them, deoxynivalenol (DON, Figure 1) is a type-B trichothecene mainly produced by *Fusarium* species that contaminates cereals and cereal-based diets (Payros et al., 2016). The European Food Safety Authority (EFSA) determined that almost half of 26,613 cereal samples collected from 21 European countries were contaminated with DON, making it the most prevalent mycotoxin contaminating human food (Knutsen et al., 2017).

Both EFSA and JEFCA (Joint FAO/WHO Expert Committee on Food Additives) established tolerable daily intake for this toxin of 1 $\mu\text{g}/\text{kg}$ body weight per day on the basis of reduced body weight gain in mice (Knutsen et al., 2017). Analyses of urine samples indicated that 40 to 90% of individuals are exposed to DON (De Santis et al., 2019; Turner et al., 2008). Hence, consumer' exposure to DON has become of particular concern, as the Tolerable Daily Intake (TDI) may be exceeded in some groups of people (Knutsen et al., 2017; Vin et al., 2020).

The intestine, especially the jejunum, has been identified as the main absorption site of DON, and the first tissue targeted by the toxin (Payros et al., 2016; Pinton & Oswald, 2014). DON has also been quantified in the ascending and transverse colon after oral exposure (Gajęcka et al., 2018) but few studies have investigated the effect of DON in the distal part of the gastro-

intestinal tract and especially the colon. Vignal et al. showed that chronic ingestion of DON impairs intestinal homeostasis in the colon, increasing myeloperoxidase activity (MPO), disturbing colonic morphology and modifying microbiota composition (Vignal et al., 2018). Inflammatory bowel diseases (IBD) are characterized by chronic inflammation of the gastrointestinal tract especially the lower part, and by colonic tissue damage. Recently, we demonstrated that subacute exposure of rodents to DON-contaminated diet exacerbates the onset and the severity of chemically-induced colitis, suggesting that this food contaminant could be a risk factor for IBD (Payros et al., 2020).

The present study was designed to determine the *in vivo* effects of dietary exposure to DON on the colonic barrier with special emphasis on intestinal permeability, expression of tight junction, inflammatory response and apoptosis. Our results demonstrated that exposure to low levels of DON is associated with the onset of clear health-threatening events in the colon, thus identifying oral exposure to DON as a risk factor in the development of IBD.

2. Material and methods

2.1 Animals and experimental design

All the experimental procedures were carried out in compliance with European Guidelines for the Care and Use of Animals for Research Purposes and were validated by the Toxicomethique Ethics Committee and the French Ministry of Higher Education, Research and Innovation (Approval certificate B31.555.13).

Four-week-old (140-170 g body weight) male Wistar rats (Janvier Labs, Le Genest Saint Isle, France), were housed in standard laboratory cages under controlled conditions and acclimated to the in-house environment for five days. Following acclimatization, the animals were allocated a control diet or diets contaminated with DON for different times of exposure. In one experiment, animals received 2 or 9 mg.kg⁻¹ DON-contaminated diet for four weeks after weaning. In another experiment, animals received control or 9 mg.kg⁻¹ DON-contaminated diet for one, two or four weeks. The diets were manufactured at the INRA UPAE (*Unité de Préparation des Aliments*, Jouy-en-Josas, France), and formulated according to rat requirements (Supplementary Material Table S1). For the contaminated diets, purified DON (reference product: D0156 ; TLC > 98.00 %) was purchased from Sigma (Saint-Quentin Fallavier, France) and mixed with the diet to reach the required level. All the diets were checked for the absence of other mycotoxins (3- and 15-acetyldeoxynivalenol, nivalenol, fusarenon X, diacetoxyscirpenol, T-2 and HT-2 toxins, zearalenone, fumonisin B₁ and B₂, moniliformin, ergot alkaloids, aflatoxins and ochratoxins) (Supplementary Material Table S1).

Throughout the study, rats had *ad libitum* access to the diets and water. At the end of the experimental period, rats were euthanized, and intestinal tissue samples were collected for subsequent analyses.

2.2 *Ex vivo* intestinal permeability assays

Colonic fragments from rats fed control or DON-contaminated diets were mounted in Ussing chambers (Easymount, Physiologic Instruments, Hamden, USA) exposing a surface area of 0.1cm² (Riba et al., 2018). Oxygenated Krebs's solution (Sigma, Saint-Quentin Fallavier, France) thermostated at 37 °C was put on each side. Electrical resistance (R) was recorded over the 2-h period of the experiment. Fluorescein sodium salt (FSS, 376 Da, Sigma Saint-Quentin Fallavier, France) used as a marker of paracellular permeability and horseradish peroxidase (HRP, 44 kDa, Sigma Saint-Quentin Fallavier, France) used as a marker of transcellular permeability were added in the mucosal compartment at 40 µg/mL and 0.4 mg/mL concentrations, respectively. Epithelial permeability to FSS was determined by measuring fluorescence intensity at 485 nm/525 nm using an automatic Infinite M20 microplate reader (Tecan, Austria). Epithelial permeability to intact HRP was determined as previously described

(Payros et al., 2014), by an enzymatic assay for the specific HRP activities found in the serosal and mucosal compartments, using the same Infinite M20 microplate reader. Permeability was calculated as the ratio of flux to the initial concentration and expressed in cm/second.

2.3 Histology, morphological and microscopic scoring analysis

Freshly harvested colon samples were fixed in 10% buffered formalin solution and embedded in paraffin using standard histological procedures. Sections (5 μm) were stained with hematoxylin and eosin for histomorphological and intestinal morphometry (assessment of crypt depth). A lesion score (Supplementary Material Table S2) including the morphology of enterocytes, interstitial edema, and lymph vessel dilation was used to compare histological changes in different conditions as previously described (Payros et al., 2020). Crypt depth was measured randomly using a MOTIC Image Plus 2.0 ML software (Motic Instruments, Richmond, Canada).

2.4 Immunofluorescence staining

In situ cell death in the intestinal mucosa was quantified by terminal deoxynucleotidyl transferase-mediated tetramethylrhodamine-dUTP (TMR-dUTP) nick end labeling (TUNEL kit, Roche) as previously described (Payros et al., 2014). Briefly, frozen sections (5 μm) of colon were fixed with 4% paraformaldehyde and permeabilized with PBS-0.1% Triton X100–0.1% sodium citrate. Apoptotic cells were labelled using the *In situ* Cell Death Detection Kit, TMR Red as the defined concentration for 60 min at 37 °C. DNA was labelled for 1 min with TO-PRO-3 (Invitrogen) and mounted using Vectashield Mounting Medium with DAPI (4',6-diamidino-2-phenylindole) staining solution (Vector Laboratories., Burlingame, CA, USA). Images were acquired with an Olympus IX70 laser scanning confocal microscope equipped with the Fluoview software FV500. Scoring (0–4) was based on the number of TUNEL-positive cells in colonic tissues (Supplementary Material Table S3).

2.5 Immunoblotting

The expression of the tight junction proteins occludin, claudin 4, and zonula-occludens 1, and proteins involved in the two main apoptosis pathways, i.e. cleaved-caspase-3, caspase-8, caspase-9, Bax/Bak, Bcl2, cytochrome C, Fatty acid synthetase receptor (FasR) and Fas-associated death domain (FADD) was assessed on colonic tissue by immunoblotting as previously described (Luo et al., 2019). Briefly, after extraction, proteins were separated on SDS-PAGE membranes probed with primary antibodies at appropriate concentrations (Supplementary Table S4). Mouse monoclonal Ab or rabbit polyclonal anti- β -actin (Cell Signaling Technology) was used as control. Membranes were then washed and incubated with secondary antibodies obtained from Biotium (Hayward, CA) (Supplementary Table S4). Infrared fluorescence intensity of the specific bands was obtained with Odyssey Infrared Imaging System (LI-COR ScienceTec, Les Ulis, France). Protein expression was estimated after normalization calculated by the ratio of the intensity of the band of interest to that of the β -actin band.

2.6 Cytokine measurements

Distal segments of colon (0.5 cm) were collected and stored at -80 °C and tissue proteins were extracted as described previously (Payros et al., 2020). Lysates of colon were processed for ELISA using commercial ELISA kits (Duoset R&D Systems, Lille, France) to quantify tumor necrosis factor α (TNF α), interleukin-17 (IL-17), IL-1 β , IL-10, lipocalin 2 and the chemokine CXCL1 (CINC-1, rat IL-8) according to the manufacturer's instructions. Protein concentrations were determined using the BCA Assay Uptima kit (Interchim, Montluçon, France). Data are expressed as picograms of cytokine per mg of tissue protein.

2.7 MPO activity

MPO activity was measured in distal segments of colon (1 cm) as previously described (Payros et al., 2020). Protein concentrations were determined using the BCA Assay Uptima kit (Interchim, Montluçon, France). MPO is expressed in units (U) per gram of colonic protein, where 1 U corresponds to the activity required to degrade 1 μmol of hydrogen peroxide in 1 min at room temperature.

2.8 Statistical analysis

The experimental data are presented as means \pm the standard errors of the mean (SEM). Differences between the experimental groups were evaluated using one-way analysis of variance, followed by a Bonferroni post hoc test (Figure 2). A Student's t-test was subsequently used to differentiate control and DON-exposed animals (Figure 3 to 7). *P* value of < 0.05 was considered significant. All tests were performed with GraphPad Prism 8.0 (GraphPad Software, Inc, San Diego, CA).

3. Results

3.1 Dietary DON exposure alters tissue integrity in dose- and time-dependent manners.

Unlike the extensive data that have been gathered on the toxicity of DON on proximal GIT, the effects of the mycotoxin on the lower part have been poorly investigated to date (Payros et al., 2020; Vignal et al., 2018). To assess if dietary DON affects the distal intestine, we compared the morphology and lesions in the colon of male Wistar rats exposed to DON-contaminated diets at different doses and durations. Feeding 2 $\text{mg}\cdot\text{kg}^{-1}$ DON-contaminated diet to the animals for four weeks had no significant effect on weight gain, whereas the 9 $\text{mg}\cdot\text{kg}^{-1}$ DON-contaminated diet reduced food intake (Supplementary Figure S1A and S1B) and weight gain. Furthermore, both concentrations of DON induced substantial alterations in the colon with numerically greater effects at the highest dose of 9 $\text{mg}\cdot\text{kg}^{-1}$ (Figure 2A). These alterations were observed as early as two weeks after the introduction of the diet contaminated with 9 $\text{mg}\cdot\text{kg}^{-1}$ DON (Figure 2B). In our experimental conditions, one week of exposure was not sufficient to induce significant histological changes. In animals fed with DON-contaminated diet, we observed a thickening of the colonic wall as compared with control animals and several damages in colonic mucosa and epithelium (Figure 2C). These damages were mainly characterized by the presence of lymphocytic and neutrophilic infiltration and by the dilatation of lymphatic vessels in the *lamina propria* (Figure 2C). In the colonic epithelium, we observed cubic and flattened enterocytes without major damage in the crypts (Supplementary figure S2). Necrotic debris were also observed in the lumen (Figure 2C).

3.2 Dietary DON exposure increases paracellular permeability in the colon

Next, we evaluated the dietary effect of DON exposure on intestinal permeability. To this end, *ex vivo* intestinal permeability from Wistar rats fed for four weeks with control diet or the 9 $\text{mg}\cdot\text{kg}^{-1}$ DON-contaminated diet was monitored in Ussing chambers. Electrical parameters as well as transcellular and paracellular permeability of the colonic tissues were measured. Exposure to the mycotoxin significantly reduced electrical resistance (Figure 3A) associated with an increase in paracellular permeability to FSS in the colon (Figure 3B). Transcellular permeability to intact HRP was not affected (Figure 3C). Taken together, these results indicate that oral exposure to DON increases colonic paracellular permeability.

3.3 Intestinal hyperpermeability is associated with down-regulation of tight junction proteins.

To further analyze the mechanisms involved in the colonic hyper-permeability induced by DON, we examined the integrity of the sealing elements of the colonic epithelium. Using

western blot, we analyzed the expression of the tight junction proteins in the colon tissue of control rats and rats fed the 9 mg.kg⁻¹ DON-contaminated diet for 4 weeks (Figure 4).

A representative blot of the transmembrane TJ protein claudin 4, the scaffolding TJ protein ZO-1, the regulatory TJ-associated protein occludin, and actin used as loading control is shown in Figure 4A. The expression of ZO-1 and of occludin proteins was clearly downregulated in the colon following exposure to DON, whereas the expression of claudin 4 was not significantly changed (Figure 4C, 4D and 4B).

3.4 Dietary DON exposure induces apoptosis in colonic tissue.

Apoptosis in the colon was quantified by *in situ* detection of apoptotic foci using the TUNEL assay, and by western blot analysis of the proteolytic cleavage of caspase-3. We observed a significant increase in the quantity of apoptotic foci in colonic tissue of animals exposed to DON-contaminated diet compared with animals fed the control diet (Figure 5A and 5B). Likewise, exposure to DON led to a significant increase in the expression of cleaved caspase-3 in the colonic tissue (Figure 5C and 5D).

3.5 Both the extrinsic and mitochondrial pathways are involved in the DON-triggered apoptosis in colonic tissue.

To decipher the mechanisms involved in the apoptosis triggered by DON, we investigated specific markers linked with the two main apoptotic pathways. As shown in representative picture in the Figure 6A, Western blot was used to analyze and quantify the expression levels of Bax/Bak (Figure 6B), Bcl2 (Figure 6C), the ratio Bax/Bcl2 (Figure 6D), cytochrome C (Figure 6E) and caspase-9 (Figure 6F) involved in the mitochondrial pathway, while the expression of FasR (Figure 6G), the adapter protein FADD (Figure 6H), and caspase-8 to analyze the extrinsic pathway.

Concerning the intrinsic (mitochondrial) pathway, oral exposure to DON led to a significant upregulation of the pro-apoptotic markers Bax, ratio Bax/Bcl2, cytochrome C, caspase-9, whereas the anti-apoptotic Bcl2 showed a trend to downregulation (Figure 6A to 6C).

Interestingly, for the involvement of the extrinsic pathway, FasR and caspase-8 were also upregulated following oral exposure to DON, whereas the expression of adapter protein FADD was not modified (Figure 6F to 6H).

Taken together, these results indicate that both the mitochondrial pathway (intrinsic) and the death receptor pathway (extrinsic) are associated with the apoptosis induced by DON in the colonic tissue.

3.6 Dietary DON exposure enhances colonic pro-inflammatory cytokine production and MPO activity.

Finally, we analyzed the colonic tissue for the production of pro-inflammatory markers that play a critical role in the development of IBD. As shown in Figure 7, TNF α (Figure 7A) and IL-17 (Figure 7B) concentrations were increased in the colonic tissue of animals fed the DON-contaminated diet. However, CXCL1, IL-1 β , and the anti-inflammatory cytokine IL-10 levels were not affected by DON exposure (Supplementary Figure S3A to S3C).

MPO activity (Figure 7C) and lipocalin-2 (Supplementary Figure S3D) concentrations were assessed as an indicator of neutrophil infiltration and subsequent inflammation in colonic tissue. The MPO activity of the animals exposed to DON was significantly higher than in the control group (Figure 7C); a similar trend was observed for lipocalin 2 (Supplementary Figure S3D).

4. Discussion

DON is one of the most frequently occurring food contaminants; awareness has increased in recent years of the adverse effects of this toxin on intestinal integrity (Akbari et

al., 2017; Pinton & Oswald, 2014a). Several studies by our group and others described the deleterious effects of DON in the upper part of the gastrointestinal tract (Robert et al., 2017). For example ingestion of a DON-contaminated diet for four weeks was shown to increase the lesion score and to decrease villi length in the jejunum tissue of rodents and pigs (Payros et al., 2017; Pierron et al., 2018). Similarly, four hours exposure of pig jejunal explants to 10 μ M DON disrupted intestinal homeostasis with an increase in the lesion score, a decrease in villi length, an increase in inflammatory response and jejunal permeability (Alassane-Kpembé et al., 2017; García et al., 2018; Pinton et al., 2009). DON was also present in the large intestine after *per os* sub-chronic exposure at a NOAEL concentration (Knutsen et al., 2017) and we recently established that dietary DON exposure exacerbates colitis induced by DSS (Payros et al., 2020). In this later model, DON exposure alone did not induce colitis but increased the pro-inflammatory response, such as IL1 β and CXCL1 production and MPO activity, when combined with DSS (Payros et al., 2020). Using the same rodent model, in the present study, we investigated the effects of dietary exposure to DON alone on the colonic tissue, specifically inflammation, permeability and apoptosis, some features of IBD.

To mimic consumer exposure, which occurs mainly through food intake, DON was incorporated in the diet. The concentration of toxin we used are very low as they encompass the NOAEL established in several studies (Arnold et al., 1986; Morrissey et al., 1985; Sprando et al., 2005). We previously determined that these doses have limited or no impact on body weight gain (Payros et al., 2017, 2020).

Here, we showed that dietary exposure to DON alters tissue integrity, and increases the production of the pro-inflammatory cytokine TNF α . Numerous studies have reported elevated levels of TNF α in patients suffering from IBD (Reimund et al., 1996; Reinecker et al., 1993) as well as in animal models (Arrieta et al., 2009; Neurath et al., 1997). DON dietary exposure also exacerbates the secretion of IL-17 in colonic tissue and pro-inflammatory markers such as MPO activity and lipocalin 2 involved in the recruitment of neutrophils. The pro-inflammatory markers produced in the colonic tissues of animals exposed to DON lead on one hand to an alteration of tight junctions and increased permeability, and on the other to an increase in the inflammatory state in the colon. These attacks are precursors of the development of IBD and reflect the involvement of DON in the inflammatory basal state of the colonic tissue.

The intestinal barrier serves as the first line of host defense against foodborne toxins. Among actors in the intestinal barrier, intestinal epithelium is a monolayer of epithelial cells, connected by TJ proteins, which form a sealed physical barrier thereby preventing the paracellular transport of luminal antigens. Disturbance of intestinal permeability and inflammation are involved in several gut dysfunctions including IBD (Bischoff et al., 2014; Salim & Soderholm, 2011). In the present study, animals exposed to DON displayed an alteration of their colonic permeability as indicated by the decrease in electrical resistance and the increased paracellular permeability to FSS. Expression of the TJ proteins is the primary determinant of paracellular permeability (Anderson & Van Itallie, 2009) and we observed a decrease in the TJ protein ZO-1 and occludin in the colon of DON exposed rats. Reduced expression of several TJ proteins, claudin 4, ZO-1 and occludin has already been observed in the small intestine of animals exposed to higher doses of DON (Akbari et al., 2014; Bracarense et al., 2012; Pinton et al., 2009). The deleterious effects of DON on colonic permeability and the integrity of the TJ protein network contribute to weakening the integrity of the intestinal barrier, thus promoting the development of IBD.

Constant homeostatic renewal of the intestinal epithelium is a physiological phenomenon that contributes to the integrity of intestinal permeability. Both apoptotic and non-apoptotic cell shedding preserve the intestinal epithelium (Blander, 2018). An increased rate of apoptosis linked with dysregulation of the caspase activity in the enterocytes induces micro-erosion that in turn, could contribute to intestinal barrier breakdown i.e., increased intestinal permeability

and inflammation. This phenomena has been shown to be involved in the pathogenesis of IBD (Becker et al., 2013; Di Sabatino et al., 2003; Kiesslich et al., 2012). A few studies have reported the effects of DON on the apoptosis in the GI tract. An increase in the number of apoptotic enterocytes was detected in the jejunal loop exposed to DON (Cheat et al., 2015). *In vitro*, DON affected intestinal epithelial cell viability through a pro-apoptotic pathway with increased Bax induction and activation of caspase-3 (Bianco et al., 2012). In the present study, we observed a significant increase in TUNEL-positive cells in the colonic tissue of DON-exposed rat in both colonic epithelial cells and in cells of the *lamina propria*. This was associated with increased activation of the apoptosis effector cleaved-caspase-3 in the tissue. *In vitro*, studies report a pro-apoptotic effect of elevated doses of DON on the leucocytes such as macrophages and neutrophils. The activation and ribotoxic stress induce by DON are responsible for the increase of apoptosis in the immune cells (Pestka, 2010). At lower doses of DON a pro-inflammatory role with an increase of the production of pro-inflammatory cytokines and immunostimulatory effect was observed (Pestka et al., 2004). This result suggests that DON exposure may affect all types of cells in the colonic tissue. To the best of our knowledge, this is the first time DON has been seen to affect colonic tissue by inducing excessive apoptosis and hyper-permeability. In IBD, apoptosis of the intestinal epithelium is often associated with inflammation. To decipher how caspase dysregulation could trigger the DON-induced apoptosis, we focused on apoptotic mitochondrial signaling pathways. The intrinsic mitochondrial p53-dependant pathway is controlled by the Bcl-2 family, including anti- and pro-apoptotic members, whereas the extrinsic pathway is induced via the activation of cell death receptors such as TNF α or Fas receptors (Blander, 2018; Elmore, 2007). The activation of both extrinsic and intrinsic pathways leads to the activation of caspase-3 and then to apoptosis. We observed that exposure to DON led to significant upregulation of Bax/Bak, cytochrome C, caspase-9 and ratio Bax/Bcl-2, confirming the role of the intrinsic pathway in the activation of DON-induced apoptosis. Indeed, DON triggers cell damage through the induction of « ribotoxic stress » and the activation of mitogen-activated protein kinases as well as their downstream pathways p53, JunN-terminal kinase (JNK) and the nuclear factor kB (NFkB) (Lucioli et al., 2013; Zhou & Pestka, 2015; Pierron et al., 2016). Through this activation, DON modulates cell growth, triggers cell cycle arrest and apoptosis (Bensassi et al., 2012; Li et al., 2014; Ma et al., 2012). In the present experiment, FasR, FADD and caspase-8 were also upregulated in the colonic tissue of animals fed the DON-contaminated diet. This underlines the involvement of the extrinsic pathway in the apoptotic response. The increased expression of TNF α corroborates this activation due to the binding of TNF α to its own receptor and to that of FasR (Becker et al., 2013). Indeed, TNF α plays an important role in the regulation of apoptotic cell death in particular through the caspase dependent pathway (Ruder et al., 2019). Our results are in line with those of previously published papers demonstrating upregulation of the expression of TNF α in intestinal epithelial and immune cells exposed to DON *in vitro* (Adesso et al., 2017; Alassane-Kpembi et al., 2017b; Cano et al., 2013; Wang et al., 2019). These findings confirm that *in vivo*, DON-induced cell apoptosis occurs both via the mitochondrial-dependent and cell death receptor pathways.

In conclusion, this study is in line with the One Health initiative that promotes integrative approaches between environment, animal health and public health and recognizes their interconnections. It highlights the effects of dietary exposure to DON in the colon and demonstrates that the exposition to this toxin induced a health-threatening breakdown of the colonic barrier. Several effects of DON are observed in colon such as hyper-permeability, disruption of the tight junction proteins and increase of apoptosis associated with microscopic damage and inflammation. Both the extrinsic and intrinsic pathways of apoptosis are involved in the cell death in colonic tissue (Figure 8). Due to the high exposure to DON worldwide (Vin et al, 2020; Knutsen et al, 2017), this study is of particular interest and shows that DON could

contribute to the development of IBD. As we already demonstrated that DON exacerbates IBD (Payros et al, 2020), it is important to take into account the exposure to DON as a risk factor for IBD.

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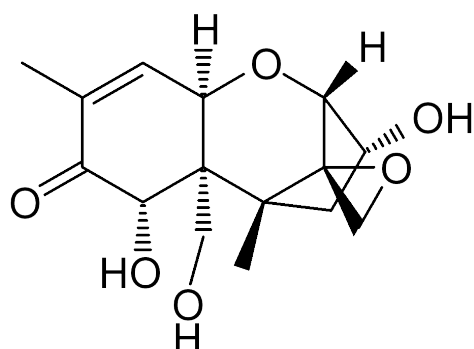


Figure 1 – Chemical structure of deoxynivalenol (DON)

Figure 2.

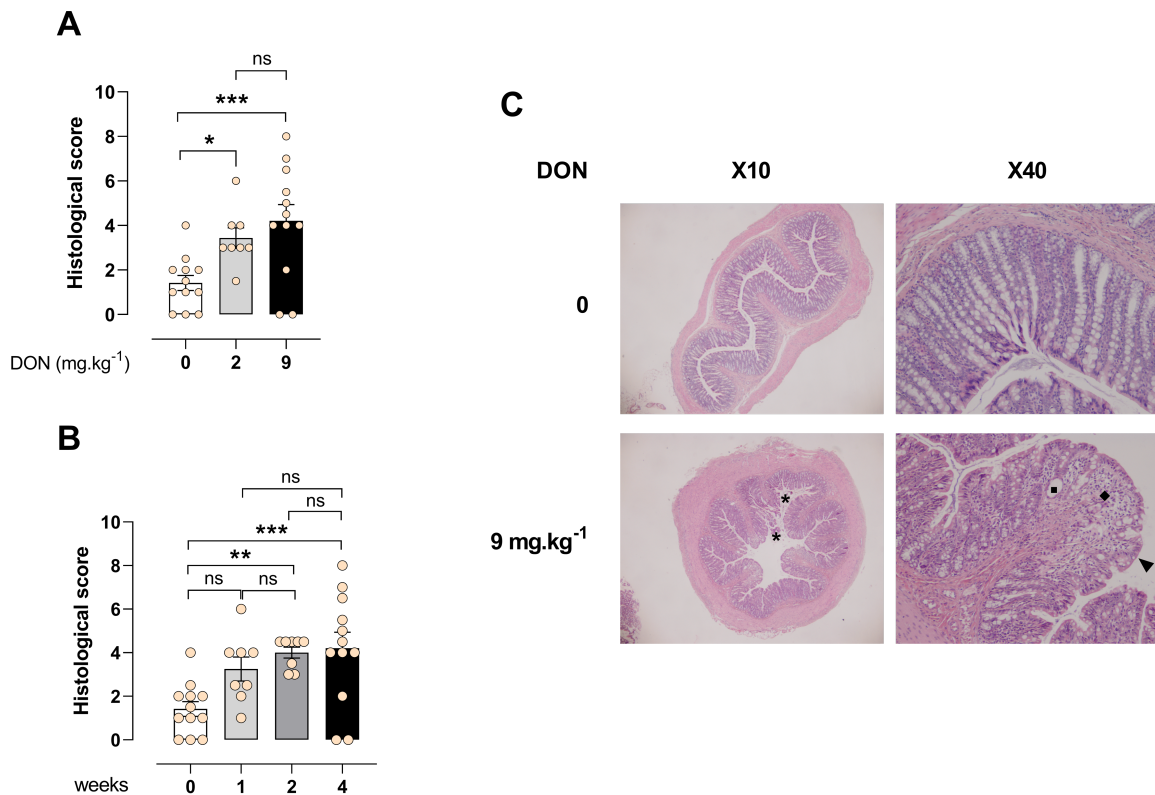


Figure 2 - Effects of DON on the colon in rats fed DON-contaminated diets at different doses or exposure times or to a control diet. (A) Wistar rats were exposed to 0, 2 or 9 mg.kg⁻¹ of DON in their diet for four weeks. (B) Wistar rats were exposed to 9 mg.kg⁻¹ of DON in their diet for 0, 1, 2 or 4 weeks. Mean histological score on the colon in a dose (A) and time (B) dependent manner. (C) Representative colonic sections with hemalun & eosin staining from rats fed the control or DON-contaminated diet (9 mg.kg⁻¹) for four weeks. (*) necrotic debris, (square) lymphatic vessel dilatation, (diamond) infiltration, (head arrow) flattened and cubic enterocytes. Mean values plus SEM are shown (n = 8 to 12 animals per condition). One-way ANOVA with Bonferroni's multiple comparison post-test. * p < 0.05; ** p < 0.01 and *** p < 0.001

Figure 3.

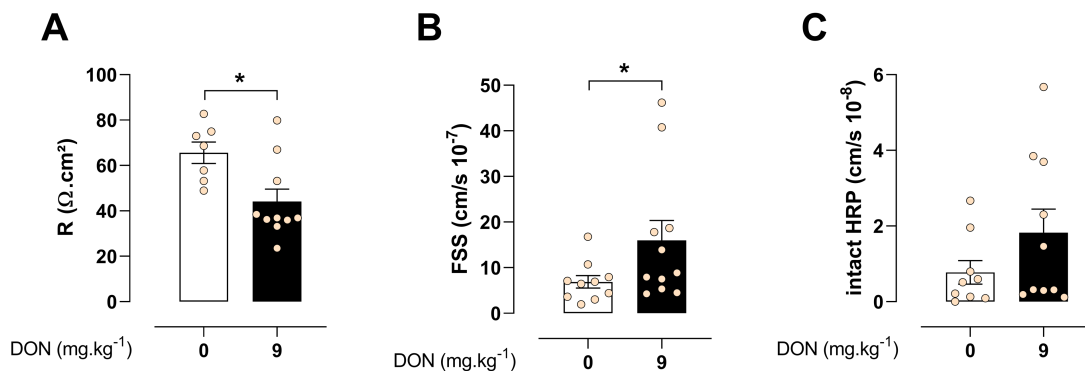


Figure 3 - *Ex vivo* permeability in the colon treated with 9 mg.kg⁻¹ of DON for four weeks. (A) Electrical resistance was recorded during the permeability analysis. (B) Paracellular permeability was assessed using mucosal to serosal flux of fluorescein sodium salt (FSS, 376 Da). (C) Transcellular permeability was assessed by measuring mucosal to serosal flux of intact horseradish peroxidase (HRP, 44KDa). Mean values plus SEM are shown (n = 7 to 10 animals per condition). Student's t-test was used for differences between control and DON-exposed animals. * p < 0.05.

Figure 4.

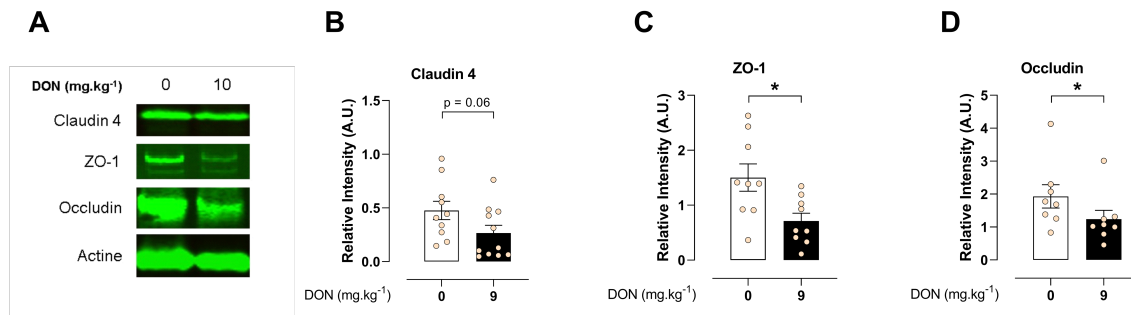


Figure 4 - Effects of DON on the expression of the tight junction proteins in colonic tissue. Rats were exposed to 9 mg.kg⁻¹ DON-contaminated diet or to a control diet for four weeks and samples of colon were analyzed by western blot for the expression of claudin-4 (B), zonula-occludens 1 (C) and occludin (D) and actin (A) used as a protein loading control. Normalized expression graphs are shown. Mean values plus SEM are shown. (n = 8 to 12 animals per condition). Student's t-test was used for differences between control and DON-exposed animals. * p < 0.05.

Figure 5.

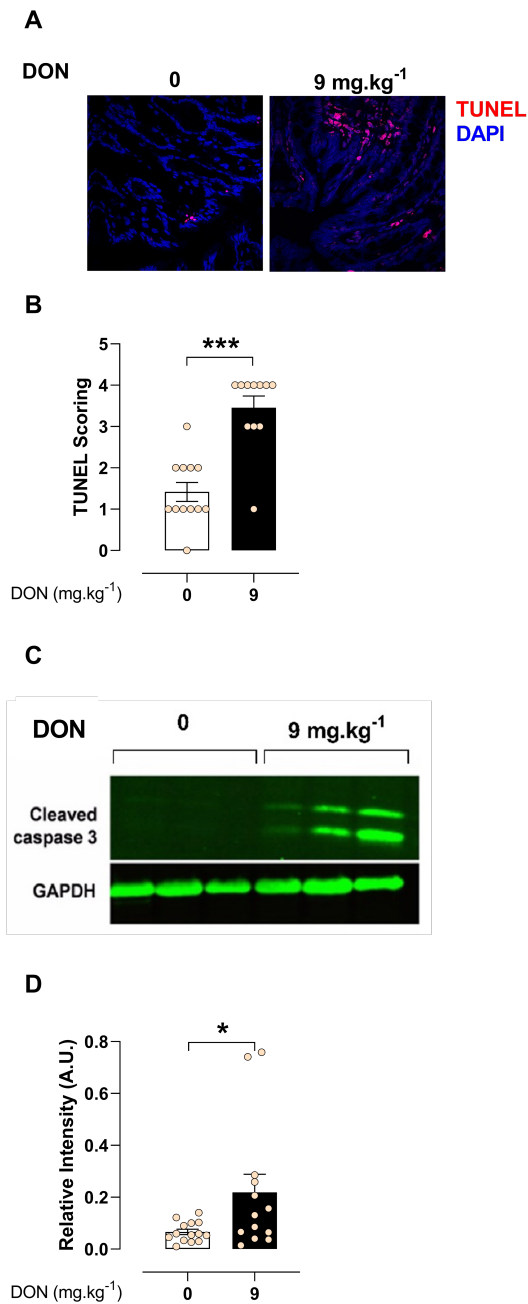


Figure 5 - Effect of DON on apoptosis in the colon. Rats were exposed to a 9 mg.kg⁻¹ DON-contaminated diet or control diet for four weeks and apoptosis was assessed in samples of colon. (A) Representative colonic frozen sections of rats fed the control or the 9 mg.kg⁻¹ DON contaminated diet for four weeks. DNA was stained blue, DAPI and apoptotic cells were stained red with anti-TdT antibody (TUNEL). (B) Quantification of intestinal apoptotic score (see Method's section). (C) Representative western blot for the expression of cleaved-caspase-3 and the protein GAPDH in the colon. (D) Quantitative levels of cleaved-caspase-3 normalized on the expression of GAPDH (used as a protein loading control). Mean values plus SEM are shown. (n = 12 to 14 animals per condition). Student's t-test was used for differences between control and DON-exposed animals. * p < 0.01 ; *** p < 0.001.

Figure 6.

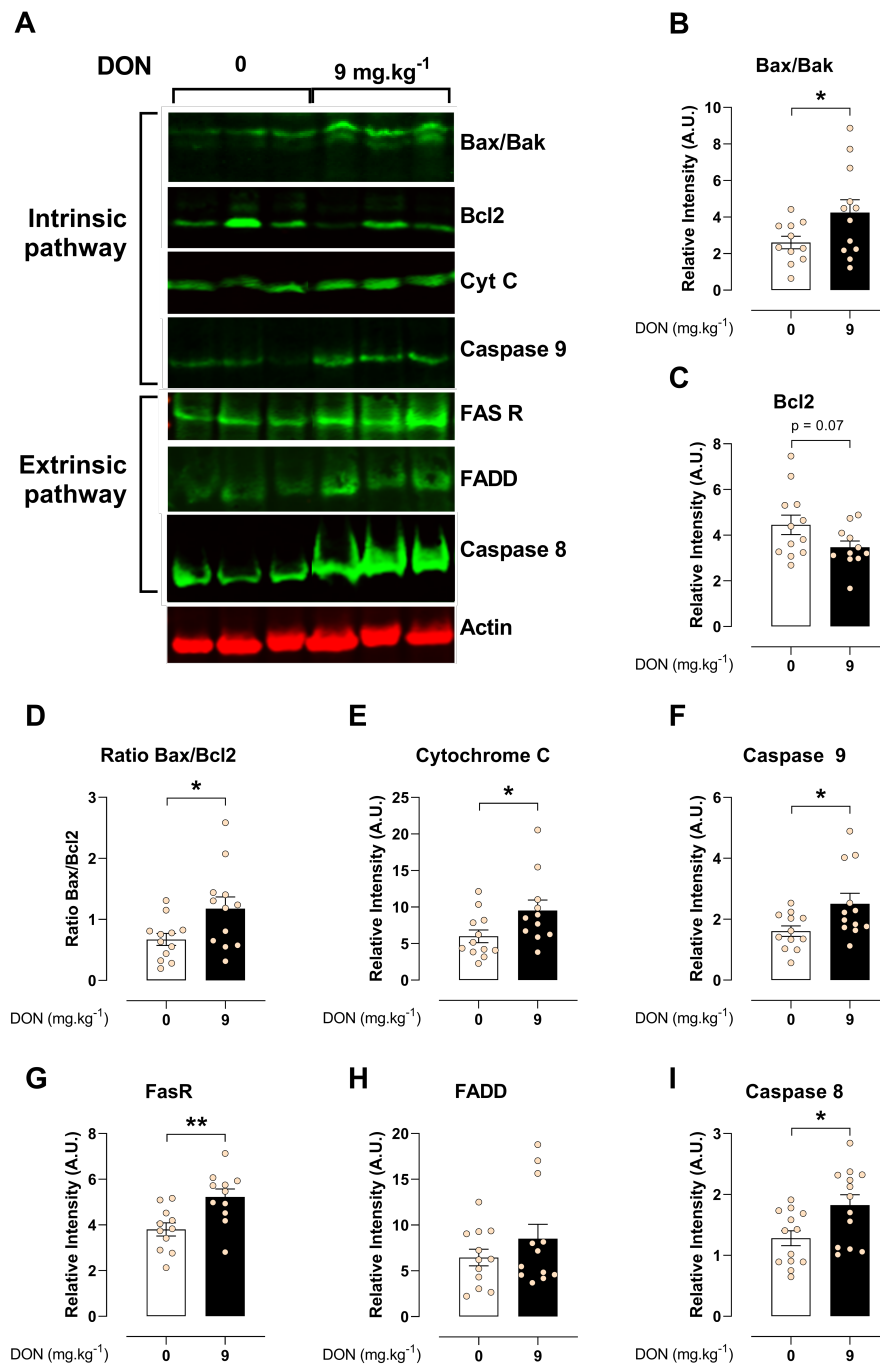


Figure 6 - Effect of DON on extrinsic and mitochondrial pathways of apoptosis in the colon. Rats were exposed to a 9 mg.kg⁻¹ DON-contaminated diet or to a control diet for four weeks. (A) Representative western blot (three animals by group) for the protein expression of intrinsic (Bax/Bak, Bcl2, Cytochrome C, Caspase 9) and extrinsic (FasR, FADD and caspase 8) pathways. The expression of actin was used as a protein loading control. Quantitative levels of Bax/Bak (B), Bcl2 (C), Ratio Bax/Bcl2 (D), cytochrome C (E), caspase-9 (F), FasR (G), FADD (H) and caspase-8 (I) normalized on the expression of actin in the colon. Mean values plus SEM are shown (n = 12 animals per condition). Student's t-test was used for differences between control and DON-exposed animals. * p < 0.05, ** p < 0.01.

Figure 7.

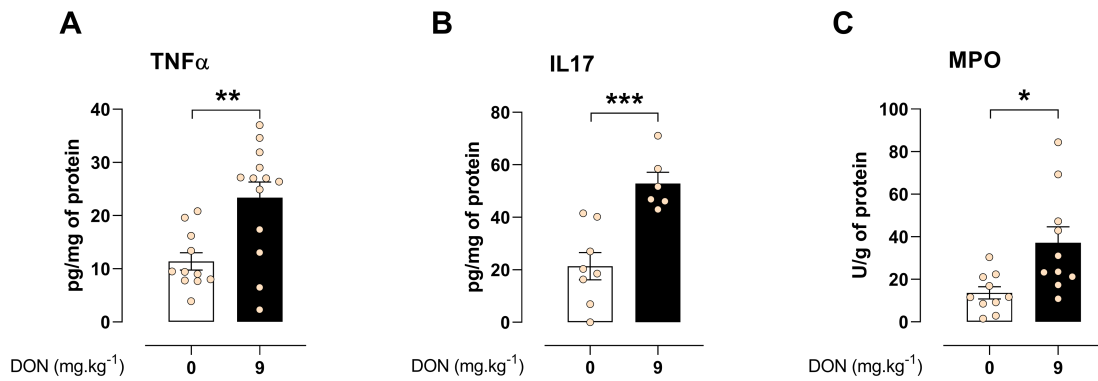
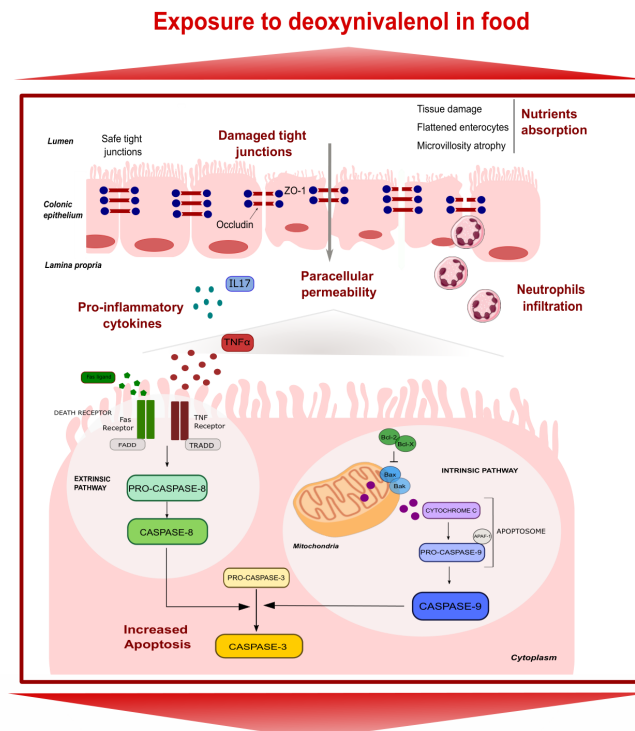


Figure 7 - Effect of DON on colonic pro-inflammatory cytokine production and myeloperoxidase activity. Rats were exposed to a 9 mg.kg⁻¹ DON-contaminated diet or to a control diet for 4 weeks and TNFα (A) and IL-17 (B) cytokine production was assessed. (C) MPO activity. Mean values plus SEM are shown. (n = 10 to 12 animals per condition for TNFα and MPO activity analyses and 6 to 8 animals for IL-17 analyses). Student's t-test was used for differences between control and DON-exposed animals. * p < 0.05. ** p < 0.01. *** p < 0.001.



**Is a risk factor to develop
Inflammatory Bowel Diseases**

Figure 8 - Dietary exposure to the food contaminant deoxynivalenol is a risk factor in triggering IBD