# Specific intestinal toxicity of deoxynivalenol and cadmium: analysis on pig jejunal explants

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SUMMARY

Deoxynivalenol (DON) is the most prevalent mycotoxin naturally present in grains and other commodities. It is produced by toxigenic fungus *Fusarium* species that are common pathogens of cereal crops under temperate climate. In pigs, chronic exposure to DON reduces feed consumption and weight gain, induces neuro-endocrine changes, and alters intestinal and immune functions. Cadmium (Cd) is one of common and widespread toxic heavy metals found naturally in the Earth's crust. Its presence in the environment is a consequence of both natural and anthropic processes. Cd can be released into soil, water and air. It is soluble in water and can be uptake by plant roots and accumulate into the edible parts especially cereal products. Chronic intoxication with Cd may result in various organs damage especially kidney. Through its high consumption of cereals, pigs can be exposed to both DON and Cd. Using pig intestinal explants this study was designed to analyze their intestinal toxicity when present alone or in combination. Jejunal explants were treated with increasing concentration of both contaminants for 4 hours and the expression of 17 genes targeting the immune response (IL-1β, IL-1α, IL-8, IL-10, IL-17a, IL-22, IFN-γ, TGF-β, TNF-α), and the oxidative stress (NF-κB, MT1A, MT2A, CCS, SOD1, SOD2, CAT, DUOX) was analyzed by RT-qPCR. Our data confirm the inflammatory effect of DON with induction of the expression of genes encoding for IL-1β, IL-1α, IL-8, and TNF-α. Exposure of pig explants to Cd induced the gene expression of metallothioneins (MT1A, MT2A) but did not have any effect on the inflammatory genes. When DON and Cd were present together, an increased expression of both inflammatory genes and metallothioneins genes was observed. Taken together our data demonstrate a specific intestinal effect of DON and Cd and suggest that these contaminants do not interact at the intestinal level.

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## INTRODUCTION

The trichothecene deoxynivalenol (DON), known colloquially as "vomitoxin", is a secondary metabolite mainly produced by the *Fusarium* genus. DON and other trichothecene mycotoxins are commonly found worldwide in cereals including wheat, barley, oat, rye, and corn, which is an increasing food safety concern in the world [Pestka, 2010; Payros et al., 2016; Placinta et al., 1999]. Human and animals are exposed to these mycotoxins by consumption of contaminated cereals leading to health problems. DON causes gastroenteritis with some primary symptoms of nausea, diarrhea and vomiting. Apart from gastroenteritis, DON exposure induces growth effects and anorexia, immune dysregulation, reproductive and teratogenic effects in experimental animals [Pestka, 2010; Smith et al., 2012].

In recent decades, amount of researches carried out on swine have demonstrated that it is the most susceptible animal to DON. The reason maybe that DON absorption is very rapid in swine and it reaches peak plasma concentration rapidly [Pestka et al., 2005; Prelusky et al., 1998]. Chronic ingestion in piglets of DON at concentration of 3 mg/kg of feed induced morphological and histological changes in jejunum, altered the gene expression of inflammatory cytokines and the serum biochemical profile and simultaneously altered the integrity of the intestinal barrier [Bracarense et al., 2012; Wu et al., 2015]. At lower concentration (0.9 mg/kg feed), a short-term exposure to piglets negatively affected weight gain, induced histomorphological alterations in the jejunum and affected, the mRNA expression of different tight junction proteins, of inflammatory and of oxidative stress marker, along the intestine [Alizadeh et al., 2015].

Cadmium (Cd) is one of the most toxic heavy metal pollutants. Due to its high mobility in soil, plant, and water systems, it is widely distributed in the environment [Manquián-Cerda et al., 2016]. The soluble form of Cd in water can be uptaken by plant roots and accumulated into the edible parts especially of cereals. The Cd concentration in crops increases with the increasing Cd concentration in soil [Jones et al., 1992; Andersson et al., 1985], causing concern for the long-term dietary intake of Cd. In addition, Cd is carcinogen [Järup & Akesson, 2009; Joseph, 2009]. Exposure to Cd can damage various organs, such as heart, lung, liver, kidney and bone. Kidney is considered to be the main target of Cd-induced toxicity [Włostowski et al., 2008; Wang et al., 2011]. The well-known effect caused by Cd is "Itai-Itai" disease in Japan [Ishihara et al., 2001].

Swine is one of the main experimental animals for the study of cadmium toxicity. Some reports have shown that there was a marked correlation between cadmium levels in porcine kidney and cadmium in feed [Wu et al., 2012; Grawé et al., 1997]. Human is at the top of food chain, therefore an increase Cd concentration in pig might be an indicator of an

increase in human Cd exposure [Grawé et al., 1997]. Kidney and liver are the two dominating organs where Cd distributes to and accumulates in, and where it exerts toxicity [Grawé et al., 1997; Hoogenboom et al., 2015; Tomović, et al., 2011]. Ingestion of food and water contaminated by Cd reduced the number of lymphocytes, affected the serum biochemical profile [Du et al., 2013].

As described above, the main source of Cd and DON exposure is the diets. Hence the intestine could be the target organ for both ingested Cd and DON [Pestka, 2010; Smith et al., 2012; Jones et al., 1992; Ninkov et al., 2015]. In addition, swine is susceptible to these contaminants. Thus, it is a good way to use a pig intestinal explants model to explore the combined toxicity of Cd and DON. This *ex vivo* model helps to reduce the number of experimental animals and preserves the normal morphological structure of intestine [Nietfeld et al., 1991]. Previous studies have used the pig jejunal explants to explore the gastrointestinal toxicity of DON [Lucioli et al., 2013; Pierron et al., 2016], and to analyze the toxicity and the interaction types of mixtures of different mycotoxins [Basso et al., 2013; Alassane-Kpembi et al., 2017].

Our aim was to analyze for the first time the toxic effects of Cd and DON, alone or in combination, as well as the interaction types between them in the intestine. We assessed the dose-dependent effect of DON and Cd on the expression of different genes by quantitative real-time polymerase chain reactions.

## MATERIAL AND METHODS

### *Animals*

Six crossbred piglets (Landrace × Large White × Duroc), 5 week-old were housed in the animal facility of the INRA ToxAlim Laboratory (Toulouse, France) and used for explants experiments and. The experimental procedures were conducted in accordance with European Guidelines for the Care and Use of Animals for Research Purposes and were approved by the Ethics Committee of Pharmacology-Toxicology of Toulouse-Midi-Pyrénées (TOXCOM/0163 PP-APAFiS#6303). Animals were fed *ad libitum* prior to the experiment.

### *Toxins*

DON and Cd were obtained from Sigma (St Quentin Fallavier, France). DON and Cd stock solutions (5 mM concentration) were prepared in sterile water and stored at −20 °C. Working dilutions were prepared in William's medium E (Sigma). The concentration range of DON and Cd were 0–24μM and 0–96 μM respectively. Explants were also exposed to a combination of DON and Cd at a ratio 1 DON/1 Cd in the range of doses 0/0 to 24/24 μM.

*Culture of explants and exposure to DON and Cd*

Jejunal explants were obtained immediately after euthanasia as previously described [25]. Fragments of 5 cm of middle jejunum were washed 4-6 times with phosphate buffered saline (PBS) and opened longitudinally with sterile scissors. Explants were collected by a biopsy punch (6 mm). Three explants were deposited, villi upward, on biopsy sponges in six-well plates containing control or DON-, Cd-, and DON/Cd-contaminated medium. All these operations were achieved in 1 h after the piglets were euthanized. Plates were incubated at 39 °C, 5% $CO_2$ for 4 h. Then explants were stored at -80 °C before transcriptional analysis

*RNA extraction*

Jejunal explants were lysed in 1 mL of Extract All reagent (Eurobio, Les Ulis, France) with ceramic beads (Bertin Technologies, St. Quentin en Yvelines, France). Total RNA was extracted as previously described [26,28]. The RNA concentration was measured by spectrophotometric analysis with Nanodrop ND1000 (Labtech International, Paris, France). The quality of RNA was determined by capillary electrophoresis with Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA, USA).

*Quantitative real-time polymerase chain reaction (RT-qPCR) analysis*

The RT-qPCR were performed using total RNA samples (n = 6 per treatment group) as previously described[26]. Non-reverse-transcribed RNA was used as non-template control for verification of absence of genomic DNA amplification signal. The specificity of the qPCR products was assessed at the end of the reactions by analyzing the dissociation curves. Primers were purchased from Sigma. The list of primers and corresponding genes is presented in table 1. Amplification efficiency and initial fluorescence were determined by LinRegPCR(2014.x) for Real Time-PCR method [29]. Then values obtained were normalized by both house-keeping genes, β2-microglobulin and ribosomal protein L32(RPL32), which were chosen with NormFinder software [30]. Finally, gene expression was expressed relatively to the control group as already described [25].

*Statistic analysis*

All data was analyzed by R program (version 3.3.2, 31.10.2016). One-way ANOVA was used to analyze the significance when data exhibited normal distribution. Otherwise, Kruskal test was used, followed by Tukey HSD (multi-comparison). A p-value $<0.05$ was considered significant. Graphs were made by GraphPad Prism 4.

Table 1. Primer sequences

| Gene symbol | Gene name | Primer sequence | Accession number and reference |
|---|---|---|---|
| IL-1β | Interleukin 1-beta | F : GAGCTGAAGGCTCTCCACCTC<br>R :TTGTTGCTATCATCTCCTTGCAC | NM_001005149[28] |
| IL-1α | Interleukin 1-alpha | F :TCAGCCGCCCATCCA<br>R :AGCCCCCGGTGCCATGT | NC_010445.3[31] |
| TNF-α | Tumor necrosis factor-alpha | F : ACTGCACTTCGAGGTTATCGG<br>R : GGCGACGGGCTTATCTGA | NM_214022[31] |
| IL-8 | Interleukin 8 | F :GCTCTCTGTGAGGCTGCAGTTC<br>R :AAGGTGTGGAATGCGTATTTATGC | NM_213867[28] |
| MT-1A | Metallothionein 1A | F :GCAAATGCACCTCCTGCAAG<br>R:CAGCCCTGGGCACACTTG | ENSSSCT00000031974.1 (This study) |
| MT-2A | Metallothionein 2A | F:CTGCAAATGCAAAGAGTGCAAA<br>R:ACACTTGGCACAGCCCACA | ENSSSCT00000029059.1 (This study) |
| RPL32 | Ribosomal Protein L32 | F :AGTTCATCCGGCACCAGTCA<br>R :GAACCTTCTCCGCACCCTGT | NM_001001636[32] |
| β2-Microglob | β2-Microglobulin | F: TTCTACCTTCTGGTCCACACTGA<br>R: TCATCCAACCCAGATGCA | NM_213978[32] |

## RESULTS

*Individual or combined effect of DON and Cd on the expression of cytokine genes*

Cytokines are very important in cell signaling and play a key role in immunomodulatory process. In order to determine the individual or combined effect of DON and Cd on the intestinal immunity, the mRNA expression of nine genes involved in inflammation (IL-1β, IL-1α, IL-8, IL-10, IL-17a, IL-22, IFN-γ, TGF-β, TNF-α) was analyzed by RT-qPCR.

The results showed that the expression levels of four inflammatory factors (IL-1β, IL-1α, IL-8, and TNF-α) were significantly elevated in the explant treated with DON at doses above 3μM (IL-1β, IL-8) or 24 μM (IL-1α, TNFα) (Fig.1). By contrast, in the Cd treatment groups, the expression levels of these genes were not affected (Fig. 1). In the explants exposed to the combination DON/Cd (ratio 1/1), the mRNA expression levels of these genes gradually increased in a dose-dependent manner, and the difference between treatment groups and control group became significant with doses higher than 6 μM, except IL-1α (Fig. 1).

In terms of genes associated with oxidative stress (NF-κB, MT1A, MT2A, CCS, SOD1, SOD2, CAT, DUOX), we only observed an increased expression of metallothioneins (MT1A, MT2A). The expression of these two genes increased with the increasing doses of Cd, and was statistically different from the control group at 24 μM. (Fig.2). By contrast, DON did not altered the expression of metallothioneins (Fig. 2). When explants were exposed to the two contaminants simultaneously, the gene expression levels increased in a dose-dependent manner, and the difference between

treatment and control groups occurred from a dose of 6 µM (ratio 1/1) (Fig.2).

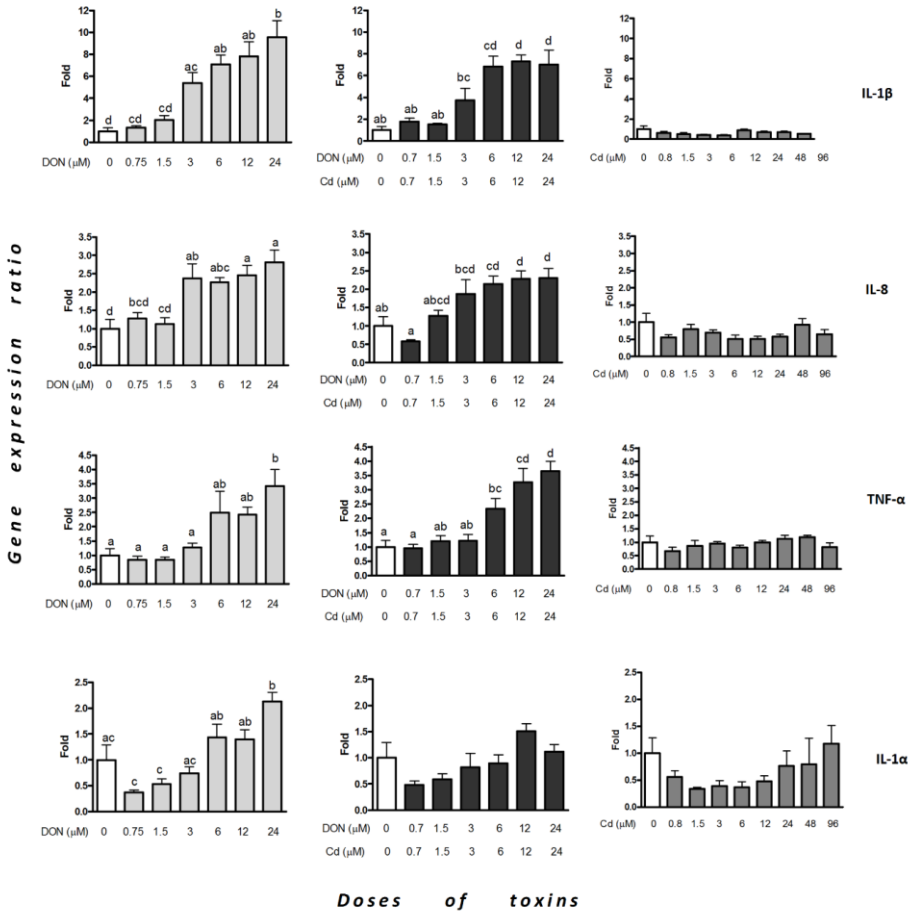


Fig.1 Dose-dependent regulation of inflammatory genes in the intestine upon exposure to DON, Cd and the co-contaminants. Jejunal explants from 6 piglets were exposed to graded levels of DON, Cd alone or in combination for 4 hours. The mRNA expression levels of IL-1β, IL-1α, IL-8, TNF-α, were measured by quantitative RT-PCR and analyzed using R program. RPL-32 and β2-microglobulin were used as the internal references. The data are the mean ± sem (n=6 animals). P-values <0.05 is considered significant.

## DISCUSSION

In the present study, we explored the combined toxicity of DON and Cd in the intestine using pig jejunal explants. Previous studies have used this model of study of the intestine to analyze the effect of cocktail of mycotoxins [Basso et al., 2013; Kolf-Clauw et al., 2013]. So far, no study using explants to investigate the toxicity of Cd or Cd combined with mycotoxins have been reported.

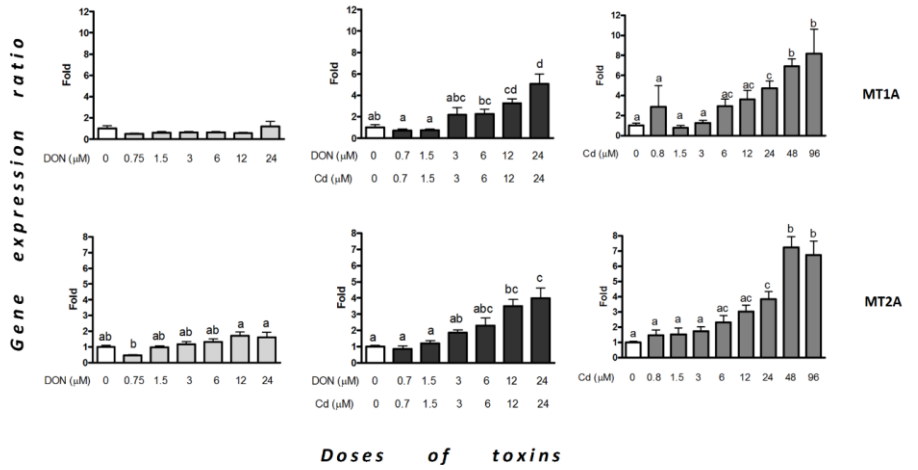


Fig.2 Dose-dependent regulation of MT genes in the intestine upon exposure to DON, Cd and the co-contaminants. Jejunal explants from 6 piglets were exposed to graded levels of DON, Cd alone or in combination for 4 hours. The mRNA expression levels of MT1A and MT2A were measured by quantitative RT-PCR and analyzed using R program. RPL-32 and β2-microglobulin were used as the internal references. The data are the mean ± sem (n=6 animals). P-values <0.05 is considered significant.

We used the RT-qPCR to detect the modulation of gene expression caused by the two contaminants. The elevation of four (IL-1β, IL-1α, IL-8, TNF-α) genes of inflammation were observed following DON exposure as already observed [Bracarense et al., 2012; Islam et al., 2006; Chung et al., 2003].

IL-8 is a chemokine, which recruits neutrophil cells and induces them to adhere to vascular endothelium and triggers extravasation into tissues [Linevsky et al., 2007]. TNF-α, IL-1β, and IL-1α are pro-inflammatory cytokines that play a key role in inflammation response. It has been reported that DON induces upregulation of pro-inflammatory genes including IL-1β, IL-6, TNF-α, MIP-2 and IL-8 through activation of MAPK [Islam et al., 2006; Chung et al., 2003]]. Therefore, we can hypothesize that the DON-induced MAPKs activation, already shown in explants [Lucioli et al., 2013] leads to an up-regulation of IL-1β, IL8, TNF-α and IL-1 α.

Metallothioneins are the markers of heavy metal toxicity and are necessary in Cd accumulation [Hoogenboom et al., 2015; Henry et al., 1994]. There are four isoforms of metallothioneins (MT-1 - MT-4), among which the MT-1 and MT-2 are the most common. They are ubiquitous in liver, intestine, pancreas and kidney [Sato & Kondoh, 2002; Werynska et al., 2015]. A decrease in metallothionein level in rat liver after exposure to moldy wheat contaminated by DON, zearalenone, T2-toxin and aflatoxins was observed. When feed was supplemented with vitamins, the levels

increased depending on the content of moldy wheat [Vasatkova et al., 2009]. To the best of our knowledge there is no report describing how DON affects the expression levels of metallothionein genes. In the present study, in the intestine exposed ex vivo, we did not observed any effect of DON on their expression at mRNA level.

MT levels and the mRNA expression levels of MT-1 and MT-2 have been found increased in pig kidney and rat mesenteric lymph node (MLN) after Cd exposure [Hoogenboom et al., 2015; Ninkov et al., 2015]. The present study corroborates the results obtained by Ninkov *et al.*

Cd exposure in rat for 30 days, could also induce mRNA up-regulation of pro-inflammatory cytokines in the intestine including TNF, IL-1β, IFN-γ, and IL-17 [Ninkov et al., 2015]. Nevertheless, in our study, Cd do not affect the expression of inflammatory cytokines genes. We can make the hypothesis that the duration of exposure of the explants was too short to observe any effect.

## Conclusions

In summary, at the level of gene expression level in the jejunal explants model, the combined effects of DON and Cd correspond to the effects of the individual compounds suggesting that these contaminants do not interact with each other.

## References

Pestka, J. J. Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. *Arch. Toxicol.* 2010, *84*, 663–679, doi:10.1007/s00204-010-0579-8.

Payros, D.; Alassane-Kpembi, I.; Pierron, A.; Loiseau, N.; Pinton, P.; Oswald, I. P. Toxicology of deoxynivalenol and its acetylated and modified forms. *Arch. Toxicol.* 2016, *90*, 2931–2957, doi:10.1007/s00204-016-1826-4.

Placinta, C. M.; D'Mello, J. P. F.; Macdonald, A. M. C. A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. *Anim. Feed Sci. Technol.* 1999, *78*, 21–37, doi:10.1016/S0377-8401(98)00278-8.

Smith, L. E.; Stoltzfus, R. J.; Prendergast, A. Food chain mycotoxin exposure, gut health, and impaired growth: a conceptual framework. *Adv. Nutr. Bethesda Md* 2012, *3*, 526–531, doi:10.3945/an.112.002188.

Pestka, J. J.; Smolinski, A. T. Deoxynivalenol: toxicology and potential effects on humans. *J. Toxicol. Environ. Health B Crit. Rev.* 2005, *8*, 39–69, doi:10.1080/10937400590889458.

Prelusky, D. B.; Hartin, K. E.; Trenholm, H. L.; Miller, J. D. Pharmacokinetic fate of 14C-labeled deoxynivalenol in swine. *Fundam. Appl. Toxicol. Off. J. Soc. Toxicol.* 1988, *10*, 276–286.

Bracarense, A.-P. F. L.; Lucioli, J.; Grenier, B.; Drociunas Pacheco, G.; Moll, W.-D.; Schatzmayr, G.; Oswald, I. P. Chronic ingestion of deoxynivalenol and fumonisin, alone or in interaction, induces morphological and immunological changes in the intestine of piglets. *Br. J. Nutr.* 2012, *107*, 1776–1786, doi:10.1017/S0007114511004946.

Wu, L.; Liao, P.; He, L.; Ren, W.; Yin, J.; Duan, J.; Li, T. Growth performance, serum biochemical profile, jejunal morphology, and the expression of nutrients transporter genes in deoxynivalenol (DON)- challenged growing pigs. *BMC Vet. Res.* 2015, *11*, 144, doi:10.1186/s12917-015-0449-y.

Alizadeh, A.; Braber, S.; Akbari, P.; Garssen, J.; Fink-Gremmels, J. Deoxynivalenol Impairs Weight Gain and Affects Markers of Gut Health after Low-Dose, Short-Term Exposure of Growing Pigs. *Toxins* 2015, *7*, 2071–2095, doi:10.3390/toxins7062071.

Manquián-Cerda, K.; Escudey, M.; Zúñiga, G.; Arancibia-Miranda, N.; Molina, M.; Cruces, E. Effect of cadmium on phenolic compounds, antioxidant enzyme activity and oxidative stress in blueberry (Vaccinium corymbosum L.) plantlets grown in vitro. *Ecotoxicol. Environ. Saf.* 2016, *133*, 316–326, doi:10.1016/j.ecoenv.2016.07.029.

Jones, K. C.; Jackson, A.; Johnston, A. E. Evidence for an increase in the cadmium content of herbage since the 1860s. *Environ. Sci. Technol.* 1992, *26*, 834–836, doi:10.1021/es00028a026.

Andersson, A.; Bingefors, S. Trends and Annual Variations in Cd Concentrations in Grain of Winter Wheat. *Acta Agric. Scand.* 1985, *35*, 339–344, doi:10.1080/00015128509442044.

Järup, L.; Akesson, A. Current status of cadmium as an environmental health problem. *Toxicol. Appl. Pharmacol.* 2009, *238*, 201–208, doi:10.1016/j.taap.2009.04.020.

Joseph, P. Mechanisms of cadmium carcinogenesis. *Toxicol. Appl. Pharmacol.* 2009, *238*, 272–279, doi:10.1016/j.taap.2009.01.011.

Włostowski, T.; Krasowska, A.; Bonda, E. Joint effects of dietary cadmium and polychlorinated biphenyls on metallothionein induction, lipid peroxidation and histopathology in the kidneys and liver of bank voles. *Ecotoxicol. Environ. Saf.* 2008, *69*, 403–410, doi:10.1016/j.ecoenv.2007.03.014.

Wang, L.; Wang, H.; Li, J.; Chen, D.; Liu, Z. Simultaneous effects of lead and cadmium on primary cultures of rat proximal tubular cells: interaction of apoptosis and oxidative stress. *Arch. Environ. Contam. Toxicol.* 2011, *61*, 500–511, doi:10.1007/s00244-011-9644-4.

Ishihara, T.; Kobayashi, E.; Okubo, Y.; Suwazono, Y.; Kido, T.; Nishijyo, M.; Nakagawa, H.; Nogawa, K. Association between cadmium concentration in rice and mortality in the Jinzu River basin, Japan. *Toxicology* 2001, *163*, 23–28.

Wu, X.; Wei, S.; Wei, Y.; Guo, B.; Yang, M.; Zhao, D.; Liu, X.; Cai, X. The Reference Dose for Subchronic Exposure of Pigs to Cadmium Leading to Early Renal Damage by Benchmark Dose Method. *Toxicol. Sci.* 2012, *128*, 524–531, doi:10.1093/toxsci/kfs173.

Grawé, K. P.; Thierfelder, T.; Jorhem, L.; Oskarsson, A. Cadmium levels in kidneys from Swedish pigs in relation to environmental factors--temporal and spatial trends. *Sci. Total Environ.* 1997, *208*, 111–122.

Hoogenboom, R. L. A. P.; Hattink, J.; Polanen, A. van; Oostrom, S. van; Verbunt, J. T.; Traag, W. A.; Kan, K. A.; Eijkeren, J. C. H. van; Boeck, G. D.; Zeilmaker, M. J. Carryover of cadmium from feed in growing pigs. *Food Addit. Contam. Part A* 2015, *32*, 68–79, doi:10.1080/19440049.2014.979370.

Tomović, V. M.; Petrović, L. S.; Tomović, M. S.; Kevrešan, Ž. S.; Jokanović, M. R.; Džinić, N. R.; Despotović, A. R. Cadmium concentrations in the liver of 10 different pig genetic lines from Vojvodina, Serbia. *Food Addit. Contam. Part B Surveill.* 2011, *4*, 180–184, doi:10.1080/19393210.2011.589035.

Du, J.; Cheng, S. Y.; Hou, W. X.; Shi, B. M.; Shan, A. S. Effectiveness of Maifanite in Reducing the Detrimental Effects of Cadmium on Growth Performance, Cadmium Residue, Hematological Parameters, Serum Biochemistry, and the Activities of Antioxidant Enzymes in Pigs. *Biol. Trace Elem. Res.* 2013, *155*, 49–55, doi:10.1007/s12011-013-9769-6.

Ninkov, M.; Popov Aleksandrov, A.; Demenesku, J.; Mirkov, I.; Mileusnic, D.; Petrovic, A.; Grigorov, I.; Zolotarevski, L.; Tolinacki, M.; Kataranovski, D.; Brceski, I.; Kataranovski, M. Toxicity of oral cadmium intake: Impact on gut immunity. *Toxicol. Lett.* 2015, *237*, 89–99, doi:10.1016/j.toxlet.2015.06.002.

Nietfeld, J. C.; Tyler, D. E.; Harrison, L. R.; Cole, J. R.; Latimer, K. S.; Crowell, W. A. Culture and morphologic features of small intestinal mucosal explants from weaned pigs. *Am. J. Vet. Res.* 1991, *52*, 1142–1146.

Lucioli, J.; Pinton, P.; Callu, P.; Laffitte, J.; Grosjean, F.; Kolf-Clauw, M.; Oswald, I. P.; Bracarense, A. P. F. R. L. The food contaminant deoxynivalenol activates the mitogen activated protein kinases in the intestine: interest of ex vivo models as an alternative to in vivo experiments. *Toxicon Off. J. Int. Soc. Toxinology* 2013, *66*, 31–36, doi:10.1016/j.toxicon.2013.01.024.

Pierron, A.; Mimoun, S.; Murate, L. S.; Loiseau, N.; Lippi, Y.; Bracarense, A.-P. F. L.; Liaubet, L.; Schatzmayr, G.; Berthiller, F.; Moll, W.-D.; Oswald, I. P. Intestinal toxicity of the masked mycotoxin deoxynivalenol-3-β-D-

glucoside. *Arch. Toxicol.* 2016, *90*, 2037–2046, doi:10.1007/s00204-015-1592-8.

Basso, K.; Gomes, F.; Bracarense, A. P. L. Deoxynivanelol and fumonisin, alone or in combination, induce changes on intestinal junction complexes and in E-cadherin expression. *Toxins* 2013, *5*, 2341–2352, doi:10.3390/toxins5122341.

Alassane-Kpembi, I.; Puel, O.; Pinton, P.; Cossalter, A.-M.; Chou, T.-C.; Oswald, I. P. Co-exposure to low doses of the food contaminants deoxynivalenol and nivalenol has a synergistic inflammatory effect on intestinal explants. *Arch. Toxicol.* 2017, *91*, 2677–2687, doi:10.1007/s00204-016-1902-9.

Ruijter, J. M.; Ramakers, C.; Hoogaars, W. M. H.; Karlen, Y.; Bakker, O.; van den Hoff, M. J. B.; Moorman, A. F. M. Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Res.* 2009, *37*, e45, doi:10.1093/nar/gkp045.

Andersen, C. L.; Jensen, J. L.; Ørntoft, T. F. Normalization of Real-Time Quantitative Reverse Transcription-PCR Data: A Model-Based Variance Estimation Approach to Identify Genes Suited for Normalization, Applied to Bladder and Colon Cancer Data Sets. *Cancer Res.* 2004, *64*, 5245–5250, doi:10.1158/0008-5472.CAN-04-0496.

Cano, P. M.; Seeboth, J.; Meurens, F.; Cognie, J.; Abrami, R.; Oswald, I. P.; Guzylack-Piriou, L. Deoxynivalenol as a new factor in the persistence of intestinal inflammatory diseases: an emerging hypothesis through possible modulation of Th17-mediated response. *PloS One* 2013, *8*, e53647, doi:10.1371/journal.pone.0053647.

Grenier, B.; Loureiro-Bracarense, A.-P.; Lucioli, J.; Pacheco, G. D.; Cossalter, A.-M.; Moll, W.-D.; Schatzmayr, G.; Oswald, I. P. Individual and combined effects of subclinical doses of deoxynivalenol and fumonisins in piglets. *Mol. Nutr. Food Res.* 2011, *55*, 761–771, doi:10.1002/mnfr.201000402.

Kolf-Clauw, M.; Sassahara, M.; Lucioli, J.; Rubira-Gerez, J.; Alassane-Kpembi, I.; Lyazhri, F.; Borin, C.; Oswald, I. P. The emerging mycotoxin, enniatin B1, down-modulates the gastrointestinal toxicity of T-2 toxin in vitro on intestinal epithelial cells and ex vivo on intestinal explants. *Arch. Toxicol.* 2013, *87*, 2233–2241, doi:10.1007/s00204-013-1067-8.

Islam, Z.; Gray, J. S.; Pestka, J. J. p38 Mitogen-activated protein kinase mediates IL-8 induction by the ribotoxin deoxynivalenol in human monocytes. *Toxicol. Appl. Pharmacol.* 2006, *213*, 235–244, doi:10.1016/j.taap.2005.11.001.

Chung, Y.-J.; Zhou, H.-R.; Pestka, J. J. Transcriptional and posttranscriptional roles for p38 mitogen-activated protein kinase in upregulation of TNF-alpha expression by deoxynivalenol (vomitoxin). *Toxicol. Appl. Pharmacol.* 2003, *193*, 188–201.

Linevsky, J. K.; Pothoulakis, C.; Keates, S.; Warny, M.; Keates, A. C.; Lamont, J. T.; Kelly, C. P. IL-8 release and neutrophil activation by Clostridium difficile toxin-exposed human monocytes. *Am. J. Physiol.* 1997, *273*, G1333-1340.

Henry, R. B.; Liu, J.; Choudhuri, S.; Klaassen, C. D. Species variation in hepatic metallothionein. *Toxicol. Lett.* 1994, *74*, 23–33.

Sato, M.; Kondoh, M. Recent studies on metallothionein: protection against toxicity of heavy metals and oxygen free radicals. *Tohoku J. Exp. Med.* 2002, *196*, 9–22.

Werynska, B.; Pula, B.; Kobierzycki, C.; Dziegiel, P.; Podhorska-Okolow, M. Metallothioneins in the lung cancer. *Folia Histochem. Cytobiol.* 2015, *53*, 1–10, doi:10.5603/FHC.a2015.0009.

Vasatkova, A.; Krizova, S.; Adam, V.; Zeman, L.; Kizek, R. Changes in metallothionein level in rat hepatic tissue after administration of natural mouldy wheat. *Int. J. Mol. Sci.* 2009, *10*, 1138–1160, doi:10.3390/ijms10031138.