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Deepoxy-deoxynivalenol retains some immune-modulatory properties of the parent molecule Deoxynivalenol in piglets

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Abstract

Deoxynivalenol (DON) is the most abundant trichothecene in food and feed. It causes both acute and chronic disorders of the human and animal intestine, liver and the immune system. The structural basis for the toxicity of DON has not been fully elucidated. Using the pig as a target and a model species for human, the toxicity of DON and its deepoxy-metabolite (DOM-1) were compared. Animals were exposed by gavage to 1 and 0.5 nmol toxin/kg bw./day for two and three weeks respectively. Whatever the dose/duration, DOM-1 was less toxic than DON in terms of weight gain and emesis. In the three week experiment, animals were vaccinated with ovalbumin, and their immune response was analyzed in addition to tissue morphology, biochemistry and hematology. DON impaired the morphology of the jejunum and the ileum, reduced villi height, decreased E-cadherin expression and modified the intestinal expression of cytokine. Similarly, DON induced hepatotoxicity as indicated by the lesion score and the blood biochemistry. By contrast, DOM-1 only induced minimal intestinal toxicity and did not trigger hepatotoxicity. As far as the immune response was concerned, the effects of ingesting DOM-1 were similar to those caused by DON, as measured by histopathology of lymphoid organs, PCNA expression and the specific antibody responses. Taken together, these data demonstrated that DOM-1, a microbial detoxification product of DON, was not toxic in the sensitive pig model but retained some immune-modulatory properties of DON, especially its ability to stimulate a specific antibody response during a vaccination protocol.

Keywords: modified mycotoxins, epoxy-group, in vivo, immune response, pig

Introduction

Mycotoxins are secondary metabolites produced by fungi. They may contaminate different food and feed commodities in many parts of the world. Among them, the trichothecene deoxynivalenol (DON), produced by *Fusarium* species, is one of the most common mycotoxins and can be found in several cereals as well as in finished food and feed. A worldwide survey indicates that DON is present in 56% of the samples tested (Schatzmayr and Streit 2013). DON is usually detected at low levels (< 1 mg/kg) and occasionally at higher levels (5 to 20 mg/kg) in cereals intended for animal or human consumption (EFSA 2017). The global occurrence and toxicity of DON are considered to be a major food safety risk for both human and animals (EFSA 2017).

The toxicity of DON is well documented; numerous studies have demonstrated its many toxic effects in humans and animals; (Maresca 2013; Pestka 2010b; Wang et al. 2014; EFSA 2017). A high concentration of DON causes abdominal distress, salivation, discomfort, diarrhea, vomiting, leukocytosis and gastrointestinal bleeding (Pestka 2010b). Chronic exposure impacts growth, immunity, intestinal barrier function and reproduction in animals (Pinton and Oswald 2014). However, the structural basis for the toxicity of DON has not been fully elucidated. Several studies suggested that the toxicity of DON is due to the integrity of its epoxy

group, the 12,13-epoxy ring (Karlovsky 2011; Payros et al. 2016; Sundstol Eriksen et al. 2004). However, a recent study demonstrates that DON and its deepoxy metabolite DOM-1 induces phosphorylation of EIF2AK2, MAPK1/3 and AKT and that DOM-1 has an impact on ovarian theca cells thereby increasing apoptosis and decreasing steroidogenesis (Guerrero-Netro et al 2017). Conversely, some DON metabolites with an intact 12,13-epoxy ring, such as the 3-epi-deoxynivalenol (3-epi-DON) and the 3-keto-4-deoxynivalenol (3-keto-DON) also display reduced toxicity (Payros et al. 2016; Pierron et al. 2016b; Shima et al. 1997). These findings show that the epoxy group only partly explains the toxicity of DON.

Among animal species, the pig is highly sensitive to DON and, upon short-term exposure, adverse effects are observed at lower levels in pig than in rodents (Pestka 2010b; Pinton and Oswald 2014). Because of its cereal rich diet, pig is potentially exposed to high levels of DON. In addition, pig can be considered as the most appropriate animal model for extrapolation to humans, as its digestive physiology is very similar to that of human (Helke and Swindle 2013).

Using purified DON and DOM-1 and the sensitive pig model, the objective of this study was to investigate the intestinal, liver and immune toxicity of these toxins. Unlike DON, DOM-1 did not induce zootechnical, intestinal and hepatic toxicity, indicating that DOM-1 is less toxic than DON. We also observed that DOM-1 retained some of the immune-modulatory properties of DON, particularly its ability to stimulate specific antibody response during a vaccination protocol.

Material and Methods

Animals, experimental design, tissue and blood sampling

All animal experimentation procedures were carried out in accordance with the European Directive on the protection of animals used for scientific purposes (Directive 2010/63/EU) and validated by Ethics Committee for Animal Experiments Toxcomethique n°86 (TOXCOM/0004/IO AMC). Forty 4-week-old weaned castrated male pigs were obtained locally (GAEC Calvignac, St Vincent d'Autejac, France). The animals were acclimatized for one week in the animal facility of the Toxalim Laboratory (Toulouse, France), prior to being allocated to different experimental groups.

Purified DON was purchased from Sigma-Aldrich (Sigma, St Quentin Fallavier, France). DOM-1 was obtained by transformation of crystalline DON (Romer Labs, Tulln, Austria), as previously described (Pierron et al. 2016b; Schwartz-Zimmermann et al. 2015). The two molecules were diluted in water and blackcurrant syrup, and administered by gavage to pigs in one shot per day as described (Grenier et al. 2012). In one experiment, the animals received 0.5 nmol toxin/b.w./day for 21 days. In the other experiment animals received 1 nmol toxin/b.w./day for 14 days.

To evaluate vaccine response, in the 21 days experiment piglets were immunized by subcutaneous injections of 1 and 2 mg ovalbumin at D3 and D8 respectively (OVA, Sigma, St Quentin Fallavier, France), as described (Meissonnier et al. 2008). Blood samples were aseptically collected from the left jugular vein at weekly intervals. The blood was collected in tubes containing sodium heparin (Vacutainer®, Becton-Dickinson, USA) for blood formula and plasma analysis. Plasma samples were obtained after centrifugation of the blood and stored at -20 °C for later analysis.

At the end of the experimental period, the animals were slaughtered by electronarcosis before exsanguination. The small intestine was removed and intestinal tissues (jejunum with and without Peyer's patches, ileum with and without Peyer's patches) were collected. Fragments of lung, liver, lymph nodes and spleen were also collected. Organ samples were fixed in 10% buffered formalin for histopathological analysis or stored at -80 °C for protein analysis or cytokine mRNA measurements. Hematoxylin-eosin (HE) staining was used for histological evaluation; periodic acid of Schiff (PAS) was used to evaluate the number of goblet cells in villi and crypts.

Biochemical parameters and antibody concentration

Plasma concentrations of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, T bilirubin, cholesterol, and total proteins were determined on a Pentra 400 chemistry analyzer (HORIBA, Kyoto) at the Anexplor Platform in Toulouse (France).

Total concentrations of the different immunoglobulin subsets were measured by ELISA as already described (Gerez et al. 2016). Antibody titers against ovalbumin were also measured by ELISA as described in (Grenier et al. 2013).

Histopathological, Immunohistochemical and Immunoblotting analysis

Histopathological analysis of fragments of lung, liver, lymph nodes, spleen and intestinal tissues was performed as already described (Grenier et al. 2012; Luciola et al. 2013). Morphometry of intestinal villous and crypt was performed using a MOTIC Image Plus 2.0 ML® image analysis system as already described (Grenier et al. 2012; Luciola et al. 2013).

Lymphocyte proliferation and apoptosis were assessed through immunohistochemical analysis of lymph nodes and spleen. Lymphocytes were immunostained for proliferating cell nuclear antigen (PCNA) expression with antibodies against PCNA (dilution 1:200, clone PC10, Zymed, Carlsbad, CA, USA). Caspase-3 (Casp-3) antibody (1:200, clone Asp 175, Cell Signalling Technology, Beverly, MA) was used to assess cell apoptosis. Immunostained cells were counted randomly in 10 fields per section at 400 magnification, as described (Gerez et al. 2016). Expression of junction proteins on jejunal samples was assessed by immunoblotting as previously described (Pinton et al. 2012).

Expression of mRNA encoding cytokines by real time PCR

Tissue RNA was extracted as already described (Grenier et al. 2012). The concentration and quality of the samples were analyzed, reverse transcription and real-time qPCR were performed as previously described (Devriendt et al. 2009) using previously published primers (Online resource, ESM_1). mRNA expression levels were expressed relative to the mean of the control group as already described (Pierron et al. 2016a).

Statistical analysis

For statistical analysis, a two way-ANOVA was performed with a Bonferroni test as post-hoc, $p < 0.05$. Gene expression data, that did not follow a normal distribution, were analyzed by Kruskal-Wallis non-parametric test. Then, in the case of significance, $p < 0.05$, Mann and Whitney test was applied.

Results

DOM-1 did not alter zootechnical parameters

The comparative effect of DOM-1 and DON was first assessed on animal zootechnical parameters. Two experiments were conducted, one using 1 nmol toxin/ kg bw./day for two weeks, and the other 0.5 nmol toxin/ kg bw./day for three weeks.

In the experiment using high doses of toxins, a significant decrease in the weight gain was observed in the DON group during the first week of exposure. In addition, four out of six pigs receiving DON vomited during the first week. By contrast, animals in the DOM-1 group had similar or even higher weight gain than control animals (Table 1). The behavior of animals was observed and filmed during gavage (Online Ressource, ESM_2). Animals in the control and DOM-1 groups were dynamic and took the gavage easily. By contrast, animals in the DON group refused the treatment and were more lethargic.

In the experiment using lower doses of toxin for three weeks, a slight decrease in weight gain was observed in the DON group during the first week of exposure and a higher weight gain was observed in the DOM-1 group during the second week of exposure to the toxin (Table 1).

DOM-1 was not toxic for the small intestine

The effects of exposure to DON and DOM-1 were investigated in several organs starting with the intestine, which is the first compartment exposed to mycotoxins. The lesions observed in the intestine were mild to moderate in all animals. The main histological changes, observed mostly in the intestine of DON treated animals, were villi atrophy and fusion with flattening of enterocytes and denuded villi (Figure 1). In both the jejunum and the ileum, with or without Peyer's patches, the lesion score observed in DON-treated animals was significantly higher than that observed in control pigs. Ingestion of DOM-1 did not induce significant histological changes in the small intestine (Figure 1). The morphology of the intestine was also evaluated by measuring the villi height. Pigs fed the DON-contaminated diet for 21 days showed a significant decrease in villi height in jejunum, whereas pigs receiving DOM-1 did not undergo significant changes in villi height in the small intestine (Figure 1).

As DON is known to alter the intestinal barrier function through an effect on junction proteins, the expression of Claudin 4, Occludin and E-cadherin was evaluated. A significant decrease in the expression of E-cadherin was observed in the jejunum of both DON and DOM-1-treated animals compared to the jejunum of controls. Whatever the treatment, no change was observed in the expression of either Occludin or Claudin 4 (Online resource, ESM_3).

The intestine also produces cytokines with pro- and anti-inflammatory properties. The comparative effect of the ingestion of DON and DOM-1 on the expression of cytokine mRNA in the jejunum is reported in Table 2. In DON treated animals, increased expression of IL8, IL10 and IL17- α mRNA was observed in the jejunum compared to control animals (3.1; 3.7 and 5.5 fold respectively). The ingestion of the toxin also significantly modulated the expression of IL1- β and IL17- α and CCL20 in jejunum with Peyer's patches compared to controls (3.2; 2.7 and 0.62 fold, respectively). By contrast, compared to the control group, DOM-1 only decreased the expression of TGF- β in jejunum with Peyer's patches (0.62 fold decrease).

Taken together, these data indicate that in comparison to ingestion of DON, ingestion of DOM-1 induced minimal intestinal toxicity as measured by histopathology, morphology (Figure 1) and cytokine expression (Table 2).

DOM-1 was not toxic for the liver

Next, the effects of DON and DOM-1 on the liver were analyzed next. As already observed in the intestine, the hepatic lesions were mild to moderate. Ingestion of DON caused the disorganization of hepatocytes, and the vacuolization and megalocytosis of the same cells (Figure 2). Animals fed DON had a significantly higher liver lesion score than control animals ($p < 0.001$, Figure 2). By contrast, the lesion score of animals fed DOM-1 was similar to that of the control animals.

Next, we investigated the effect of DON and DOM-1 on plasmatic biochemical analytes, as biomarkers of liver lesion. We observed only a significant increase in total bilirubin in animals in the DON-treated groups at day 7 compared to the control animals (Online resource, ESM_4). By contrast, ingestion of DOM-1 caused no changes in any of the biochemical parameters analyzed.

These data indicate that compared with ingestion of DON, the ingestion of purified DOM-1 induced minimal liver toxicity as measured by histopathology (Figure 2) and blood biochemistry (Online resource, ESM_4).

DOM-1 retained immunomodulatory properties

The immune system is a target for DON (Pestka 2010a; Wang et al. 2014). The last part of our toxicological investigations compared the effect of DON and DOM-1 on the histology of lymphoid organs (spleen and lymph nodes), and assessed the effect of these toxins on antibody response.

The lesions observed in the lymphoid organs were mild to moderate. Lymphoid depletion and apoptosis of lymphocytes were the most frequent changes observed in the spleen and mesenteric lymph node. With the expression of caspase-3, apoptosis increased significantly in presence of DON in both organs (data not shown). A reactive germinal center with macrophages containing tangible bodies were also observed in the spleen of animals in the DON treated group (Figure 2). A significant increase in the lesion score was observed in the mesenteric lymph nodes and the spleen of animals treated with DON compared to control group (Figure 2). The impact of DOM-1 on lymphoid organs was similar to that of DON with increased histological lesions in the mesenteric lymph nodes and the spleen. Both DON and DOM-1 significantly increased the number of proliferating cells, as measured by PCNA expression, compared with the control group (Figure 3, panel A).

The effects of DON and DOM-1 on total and specific antibody responses were also analyzed. The plasma concentration of total IgG and IgA was not affected by exposure to DON, but the toxin slightly decreased the level of IgM compared to that in control animals (Online resource, ESM_5). The immunization protocol enabled investigation of the effects of mycotoxins on antigen-specific immunity. Ingestion of DON significantly increased the production of vaccine specific antibody compared to non-exposed animals (Figure 3, panel B). The impact of ingestion of DOM-1 was similar to ingestion of DON on specific antibody production, with a significant increase in OVA specific IgG (Figure 3, panel B).

Taken together, these data show that the effect of ingestion of purified DOM-1 was similar to that of ingestion of DON as on the immune response as measured by histopathology of the lymphoid organs (Figure 2), the PCNA expression (Figure 3, panel A) and the specific antibody response (Figure 3, panel B).

Discussion

The aim of the current study was to gain more knowledge concerning the structural basis of the toxicity of DON. To this end, the *in vivo* toxicity of DON and its deepoxy-form, DOM-1 was compared. The data presented in this paper clearly show that deepoxydation of DON strongly reduced its effect on weight gain, emesis as well as its liver and intestinal toxicity. By contrast, despite of the loss of the group epoxy bound, DOM-1 retained some immune-modulatory effects of DON, especially its adjuvant activity.

In the present study, piglets were exposed to 1 or 0.5 nmol DON or DOM-1 /kg bw./day for two or three weeks, respectively. These concentrations of DON induced toxicity (Pestka 2010b), and could thus be used to compare the effects of equimolar concentrations of DOM-1. Based on average consumption of feed by pigs at this age, these doses correspond to feed contaminated at a concentration of 3 to 9 mg of DON /kg. Weight gain was assessed in animals exposed to DON or DOM-1. Over the 14 day exposure period, lower weight gain was observed in animals treated with 1 nmol/kg bw/day DON in particular at week one. These results are in agreement with those of numerous studies describing decreased feed intake and decreased weight gain upon DON consumption (EFSA 2017; Pestka 2010b). No effect on weight gain was observed in animals exposed to DOM-1. To the best of our knowledge, this is the first *in vivo* experiment using purified DOM-1. However several studies using DON-contaminated feed in the presence of a bacterial strain able to de-epoxidise DON into DOM-1 have been performed (Li et al. 2011) and showed no effect of DOM-1 on feed intake and weight gain in comparison to the control group.

In this study, we also compared the intestinal toxicity of DON and DOM-1. The gastrointestinal tract is the first barrier against feed contaminants, and following ingestion of contaminated food or feed, intestinal epithelial cells can be exposed to a high concentration of toxicants, potentially affecting intestinal functions

(Akbari et al. 2014; Ghareeb et al. 2015). DON is known to target the intestine (Payros et al. 2016; Pinton and Oswald 2014). The present study confirmed that DON significantly decreases jejunal villi height, increases apical necrosis, induces mild to moderate intestinal tissue lesions including villi atrophy and fusion and reduces the expression of E-cadherin (Bracarense et al. 2012; Ghareeb et al. 2015). Similar changes have also been observed in intestinal explants treated with the toxin (Alassane-Kpembi et al. 2017a; Luciooli et al. 2013; Pierron et al. 2016a). DON, like other trichothecenes, alters the local immune response (Pestka 2010a; Payros et al. 2016). In the present experiment, we observed increased expression of IL8, IL1- β , IL17- α , and IL10 in the jejunum of DON treated animals. Similar inflammatory expression in the presence of DON has already been observed in human and porcine enterocytes as well as in porcine explants (Alassane-Kpembi et al. 2017b; Maresca et al. 2008; Van De Walle et al. 2010). The activation of pro-inflammatory cytokines has also been described in piglets exposed to 3 mg DON/kg feed (Bracarense et al. 2012).

The effects of DOM-1 on the intestine are poorly documented and only *in vitro* and *ex vivo* data are available. The *in vitro* data mainly concern its effect on intestinal cell lines used to assess its effect on cell viability. In that study the *in vitro* cytotoxicity of DOM-1 was assessed on the intestinal cell lines IPEC-1 and IPEC-J2 with a WST-1 assay. In addition to interleukin, nitric oxide (NO) and albumin release were assessed with no observed effects of DOM-1 up to 228 μ M (Mayer et al. 2017). In another study, a CTG assay showed that, at a concentration of 10 μ M, DOM-1 was not cytotoxic, did not affect oxygen consumption and was unable to induce MAPKs activation in Caco-2 cells in accordance with the theory that DOM-1 is unable to induce ribotoxic stress like DON. Moreover, the barrier function assessed through TEER measurement shows that at a concentration of 30 μ M, the barrier function is not impaired (Pierron et al. 2016b). DOM-1 does not affect IPEC-J2 cells in any of several cytotoxicity tests and for all the parameters investigated (induction of apoptosis, MAPK signaling, alteration of mitochondrial structure and function) at concentrations up to 100 μ M (Springler et al. 2017). In agreement with *in vitro* results, in the present study, we observed that ingestion of DOM-1 did not cause intestinal damage, as assessed by the villi morphometry and the lesion scores in different intestinal segments. What is more, DOM-1 did not increase pro-inflammatory cytokines in the intestinal tract. These results are also in accordance with those found in jejunal pig explants in the presence of 10 μ M DOM-1, with no evidence of intestinal lesions or changes in cytokine gene expression (Pierron et al. 2016b).

Signs of hepatotoxicity were observed upon ingestion of DON in accordance with previous reports (Mikami et al. 2010). DON has been shown to act on the liver of both laboratory and farm animals, and to affect structural integrity and functions. Microscopic changes observed in the present experiment have already been described in the literature, including disorganization, vacuolation, and megalocytosis of the hepatocytes (Grenier et al. 2013; Mayer et al. 2017). Similarly, biochemical changes are reported in pigs after DON ingestion (Grenier et al. 2013; Mayer et al. 2017; Pinton et al. 2012). In the present study, only one biochemical marker (total bilirubin), showed a transient increase in animals exposed to DON. In contrast to DON, DOM-1 did not induce any hepatotoxicity. *In vitro*, the albumin secretion by HepG2 cells was shown to decrease at a dose of 228 μ M of DOM-1 concentration (Mayer et al. 2017).

In the last part of this study, the effects of DON and DOM-1 on the immune response were compared. Both DON and DOM-1 induced lesions in lymphoid organs such as the spleen and lymph nodes. These toxins also increased PCNA expression in the lymph nodes. Similar effects were reported in poultry exposed to DON ((Chen et al. 2017; Girish et al. 2010). In the present study, an increase in the specific immune response against ovalbumin was observed in both DOM-1 and DON treated animals compared to control pigs. Concerning DON, similar results were already observed in pigs, in which DON stimulated the specific immune response (Pinton et al. 2008). The effect of DOM-1 on the immune response has never been investigated *in vivo*. An *in vitro* study reports an inhibitor effect of DOM-1 on the proliferation of porcine and chicken peripheral blood mononuclear cells (Novak et al. 2018). Our results indicated that DOM-1 induced also some *in vivo* effects, i.e., a reactive germinal center with macrophages in the spleen, an increase in cell proliferation in lymph nodes and stimulation of the specific immune response. However, DOM-1 did not induce apoptosis in the spleen and lymph nodes (data not shown), nor toxic effects in liver and intestinal tissue and zootechnical decrease. DOM-1 thus seems to act as an adjuvant, triggering more specifically the humoral immune response (Pestka 2010a).

One aim of this study was to describe the structural basis of the toxicity of DON. This toxin is known to have several effects including anorexia, apoptosis, hepatotoxicity and alteration of intestinal functions. These effects are linked with the induction of ribotoxic stress and activation of MAPKs (Liao et al. 2018; Payros et al. 2016). We previously demonstrated that DOM-1 did not activate MAPKs (Pierron et al. 2016b) and the data presented here showed that this toxin did not show hepatic and intestinal toxicity, apoptosis in lymph nodes nor increase the expression of pro-inflammatory cytokines in the intestine. This suggests that the epoxy group is involved in the induction of ribotoxic stress and the ribosome dependent MAPK signaling pathways. The present study also demonstrated that DOM-1 retained some immunological properties, especially an adjuvant capacity for antibody response. It has recently been shown that DOM-1 stimulates phosphorylation of EIF2AK2, MAPK3/1, and MAPK14 and increases the mRNA level of the endoplasmic reticulum (ER) stress-related proteins, PRKRA and AFT4 (Guerrero-Netro et al. 2017). We can thus hypothesize that DOM-1 targets the

lymphoid organs and the specific immune response, without involving the ribosome, via alternative mechanism involving ER-stress.

In conclusion, this study demonstrated that ingestion of DOM-1 results in strongly reduced toxicity compared to ingestion of DON. Absence of toxicity has already been described in pigs and poultry receiving DON contaminated feed supplemented with a feed additive or with bacteria able to de-epoxidize the DON (Awad et al. 2014; Ghareeb et al. 2012; Grenier et al. 2013; Li et al. 2011). The present study increases this knowledge, as feeding animals with purified DOM-1 and demonstrating that DOM-1 failed to induce apoptosis on lymphoid organs, or have a toxic effect on the intestine (lesions, inflammatory reaction) or in the liver. Our results also demonstrate that DOM-1 retains some immune-modulatory properties of DON, especially its ability to stimulate the specific antibody response during a vaccination protocol. Further studies are needed to understand the mechanism of action of DOM-1, and especially its possible adjuvant capacity.

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Table 1: Comparative effects of DOM-1 and DON on animal performance

Toxin exposure (dose, duration)	Weight gain assessment	Animal group		
		Control	DON	DOM-1
1 nmol/kg bw/day for 14 days				
	<i>Week 1</i>	5.37 ± 0.88 ^b	3.95 ± 0.68 ^a	5.78 ± 0.33 ^b
	<i>Week 2</i>	4.45 ± 0.71 ^{ab}	4.20 ± 0.88 ^b	5.27 ± 0.80 ^a
0.5 nmol/kg bw/day for 21 days				
	<i>Week 1</i>	3.21 ± 0.56 ^a	2.89 ± 0.86 ^a	3.25 ± 0.56 ^a
	<i>Week 2</i>	4.51 ± 0.76 ^a	4.65 ± 0.55 ^a	5.15 ± 0.59 ^a
	<i>Week 3</i>	6.94 ± 0.93 ^a	7.01 ± 1.23 ^a	6.98 ± 0.95 ^a

Animals were exposed daily to DON, DOM-1 or diluent by gavage and their weight gain was recorded weekly. Data are expressed as mean weekly weight gain per piglet ± SD for 6 to 8 animals. Means in a line with different letters differ ($p < 0.05$).

Table 2: Comparative effect of DOM-1 and DON on intestinal inflammatory response.

Cytokine	Jejunum			Jejunum with Peyer's patches		
	Control	DON	DOM-1	Control	DON	DOM-1
IL1-β	1 ± 0.20 ^a	0.75 ± 0.13 ^a	0.67 ± 0.16 ^a	1 ± 0.20 ^a	3.24 ± 0.85 ^b	1.27 ± 0.22 ^a
IL6	1 ± 0.31 ^a	1.05 ± 0.28 ^a	1.25 ± 0.37 ^a	1 ± 0.13 ^a	1.5 ± 0.48 ^a	1.05 ± 0.13 ^a
IL8	1 ± 0.13 ^a	3.08 ± 0.78 ^b	0.78 ± 0.23 ^a	1 ± 0.06 ^a	1.97 ± 0.40 ^a	1.07 ± 0.13 ^a
IL1-α	1 ± 0.09 ^a	1.37 ± 0.26 ^a	0.96 ± 0.21 ^a	1 ± 0.12 ^a	1.63 ± 0.26 ^a	1.11 ± 0.19 ^a
IL10	1 ± 0.26 ^a	3.73 ± 1.38 ^b	1.19 ± 0.47 ^a	1 ± 0.24 ^a	2.01 ± 0.83 ^a	0.87 ± 0.25 ^a
IL17-α	1 ± 0.20 ^a	5.53 ± 1.90 ^b	0.93 ± 0.48 ^a	1 ± 0.28 ^a	2.67 ± 0.82 ^b	0.99 ± 0.25 ^a
IFN-γ	1 ± 0.24 ^a	0.84 ± 0.24 ^a	0.77 ± 0.31 ^a	1 ± 0.26 ^a	1.75 ± 0.44 ^a	1.03 ± 0.23 ^a
TNF-α	1 ± 0.13 ^a	0.77 ± 0.09 ^a	1.04 ± 0.22 ^a	1 ± 0.16 ^a	0.77 ± 0.37 ^a	1.03 ± 0.34 ^a
TGF-β	1 ± 0.29 ^a	0.52 ± 0.29 ^a	1.34 ± 0.39 ^a	1 ± 0.21 ^a	0.95 ± 0.34 ^a	0.62 ± 0.21 ^b
CCL20	1 ± 0.34 ^a	0.67 ± 0.21 ^a	0.50 ± 0.09 ^a	1 ± 0.23 ^a	0.62 ± 0.21 ^b	0.69 ± 0.21 ^a

Animals were exposed to 0.5 nmol toxin/Kg BW or diluent by gavage for 21 days. At the end of the experiment, samples of jejunum with or without Peyer's patches were collected, and mRNA levels of inflammatory markers (IL1-β, IL6, IL8, IL1-α, IL10, IL17-α, IFN-γ, TNF-α, TGF-β, CCL20) were measured by RT-PCR. Results are expressed as the relative mRNA expression as means ± SEM; n=6 to 8 animals/group. Means for an organ in a line with different letters differ ($p < 0.05$).

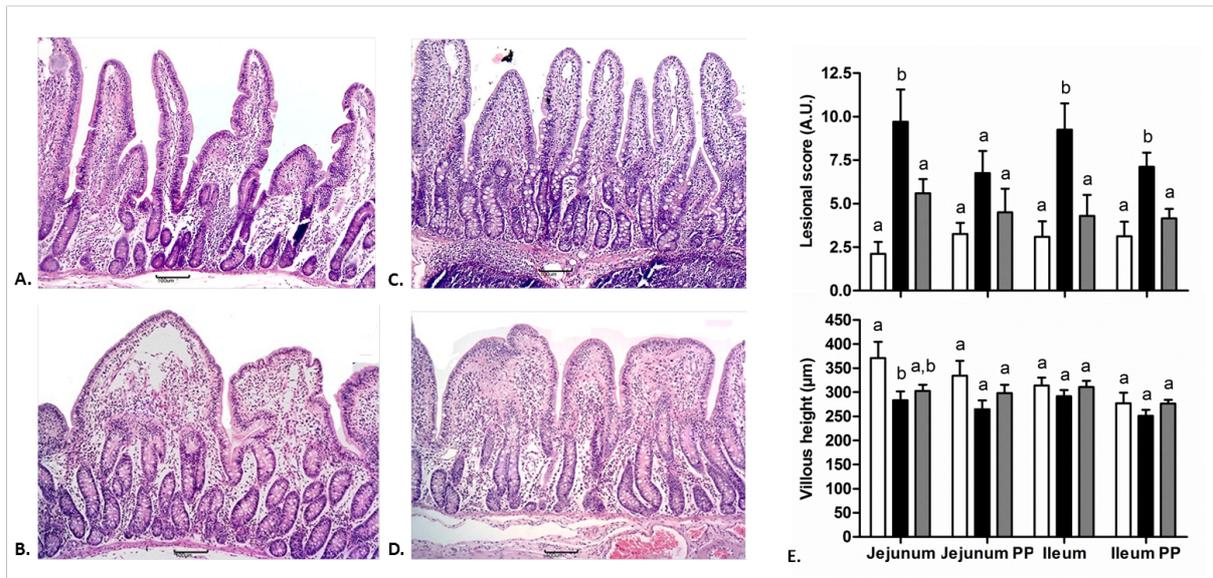


Figure 1: Histopathological effects of DON and DOM-1 on the intestine.

Piglets were exposed to 0.5 nmol/kg bw/day of DON (black bars), DOM-1 (grey bars) by gavage for 21 days or used as control animals (white bars). (A) Jejunum from control group. Villi with normal morphology. (B) Jejunum from DON group. Villi fusion and lymphatic vessel expansion. (C) Jejunum with Peyer patches from DOM-1 group. Villi with normal morphology. (D) Ileum from DON group. Villi atrophy, villi fusion and edema of lamina propria. Hematoxylin-eosin. Bar 100 µm.

Lesion scores were established after histological examination according to the severity and extent of the lesions. Values are means ± SEM of 6 to 8 animals. Graphic bars with different letters differ, $p < 0.05$.

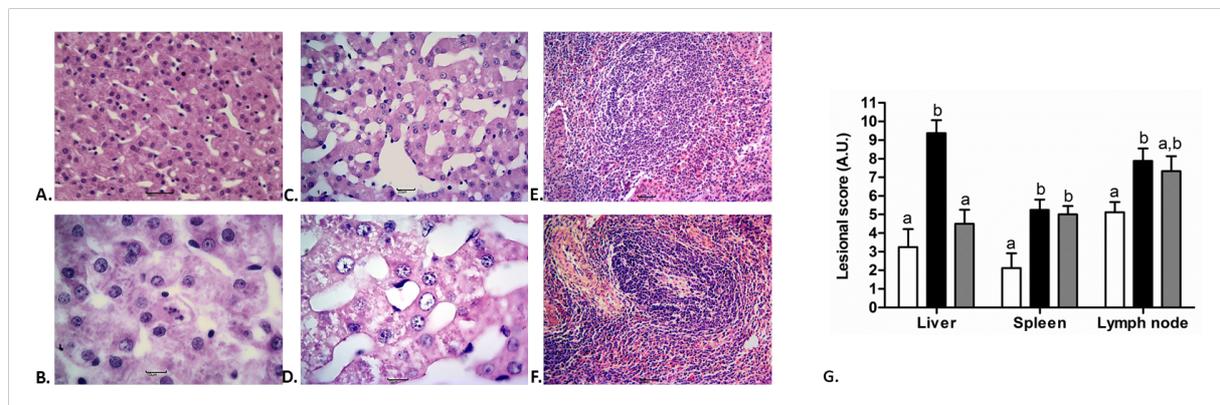


Figure 2: Histopathological effects of DON and DOM-1

Piglets were exposed to 0.5 nmol/kg bw/day of DON (black bars), DOM-1 (grey bars) by gavage for 21 days or used as control animals (white bars). A- Control group. Hepatocyte trabeculae with normal arrangement. B- DON group. Hepatocyte apoptosis (arrows) and mild vacuolation of hepatocytes cytoplasm. C- DOM-1 group. Disorganization of hepatocyte trabeculae and mild vacuolation of cytoplasm. D- DON group. Nuclear vacuolation of hepatocyte (arrow) and megalocytosis. Hematoxylin-eosin. Bar 30 µm (A), 20 µm (C), 10 µm (B,D). E- Spleen, control group. Lymphoid follicle with normal arrangement. F- Spleen, DON group. Reactive germinal center with macrophages containing tangible bodies (arrow). Hematoxylin-eosin. Bar 40 µm (A), 50 µm (B).

Lesion scores were established after histological examination according to the severity and extent of the lesions. Values are mean ± SEM for 6 to 8 animals. Graphic bars with different letters differ ($p < 0.01$).

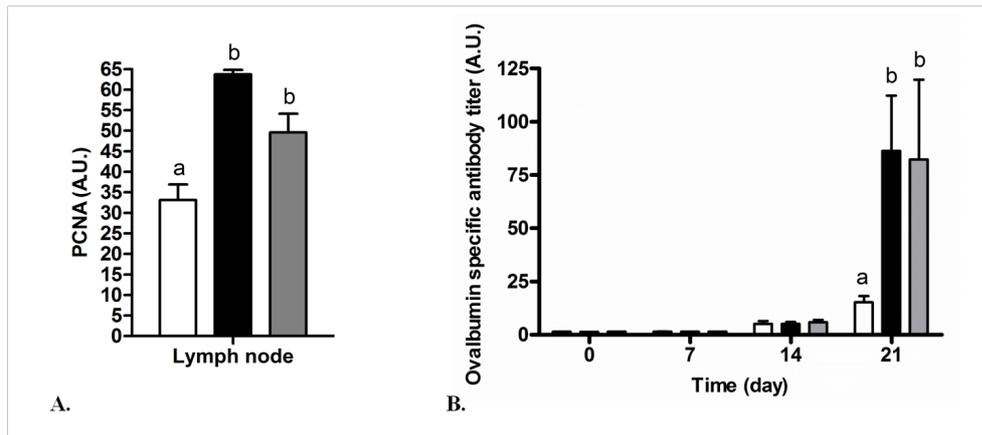


Figure 3: Effect of DON and DOM-1 on PCNA expression in lymph nodes and on the specific antibody response.

Piglets were exposed to 0.5 nmol/kg bw/day of DON (black bars), DOM-1 (grey bars) by gavage for 21 days or used as control animals (white bars). Values are means \pm SEM for 6 to 8 animals. Graphic bars with different letters differ ($p < 0.01$).

Panel A: Sections of embedded tissues were immunostained for PCNA. The total number of PCNA positive cells was counted in 10 fields in each section displayed randomly on the monitor as a high-power view.

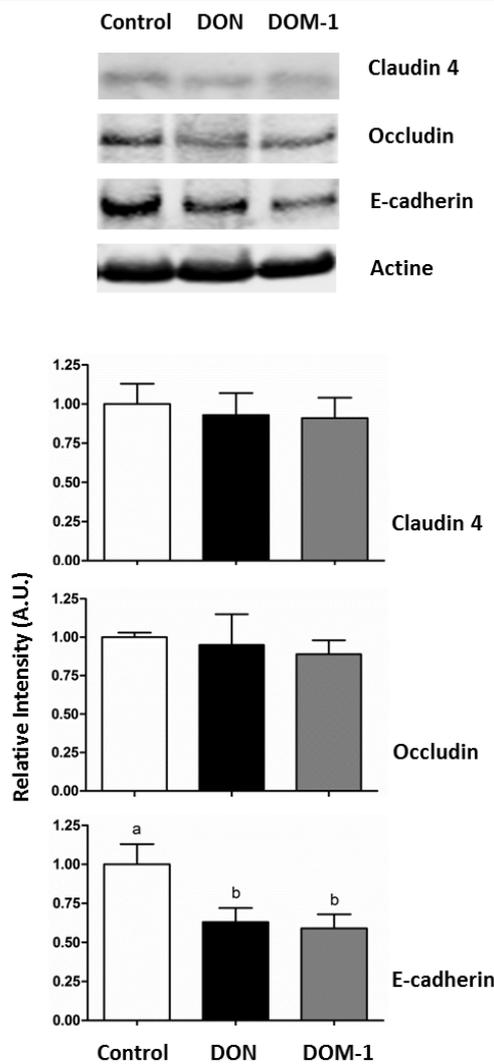
Panel B: At days 3 and 8 of the trial, all animals were subcutaneously immunized with OVA. Plasma samples were collected weekly and the level of IgG specific for OVA was determined by ELISA and normalized against standardized reference plasma.

ESM 1 : Table of primer sequences used for RT-qPCR analysis (F: Forward; R: Reverse)

Gene symbol	Gene name	Primer sequence	References
CycloA	Cyclophilin A	F: CCCACCGTCTTCTTCGACAT R: TCTGCTGTCTTTGGAACCTTTGTCT	NM_214353 Pinton et al 2015
IL1- β	Interleukin 1 beta	F: ATGCTGAAGGCTCTCCACCTC R: TTGTTGCTATCATCTCCTTGAC	NM_214055 Von der Hardt et al.2004
IL6	Interleukin 6	F: TTCACCTCTCCGACAAAACCTG R: TCTGCCAGTACCTCCTTGCTGT	NM_214399 Grenier et al. 2011
IL8	Interleukin 8	F: GCTCTCTGTGAGGCTGCAGTTC R: AAGTGTGGAATGCGTATTTATGC	NM_213867 Cano et al. 2013
IL1- α	Interleukin 1 alpha	F: TCAGCCGCCATCCA R: AGCCCCGGTGCCATGT	NM_214029,1 Cano et al. 2013
IL10	Interleukin 10	F: GGCCCAGTGAAGAGTTTCTTTC R: CAACAAGTCGCCATCTGGT	NM_214041 Cano et al. 2013
IL17- α	Interleukin 17 alpha	F: CCAGACGGCCCTCAGATTAC R: CACTTGGCCTCCAGATCAC	AB102693 Cano et al. 2013
IFN- γ	Interferon gamma	F: TGGTAGCTCTGGGAAACTGAATG R: GGCTTTGCGCTGGATCTG	NM_213948 Cano et al. 2013
TNF- α	Tumor necrosis factor alpha	F: ACTGCACTTCGAGGTTATCGG R: GGCGACGGGCTTATCTGA	NM_214022 Cano et al. 2013
TGF- β	Transforming growth factor beta	F: GAAGCGCATCGAGGCCATTC R: GGCTCCGGTTCGACACTTTC	NM_214015 Cano et al. 2013
CCL20	Chemokine (C-C motif) ligand 20	F: GCTCCTGGCTGCTTTGATGTC R: CATTGGCGAGCTGCTGTGTG	NM_001024589 Cano et al. 2013

ESM_2 : Videos of the piglets during a gavage of the different treatments at day 7.

The videos show the gavage and the behavior of animals from each group at day 7 of the trial. The treatment was administered by gavage for 14 days, at the same molar concentration (1 nmol/kg BW/ day). Toxins were diluted in water and blackcurrant syrup, to increase the palatability. The control group received only water and blackcurrant syrup.



ESM_3 : Figure of the effect of DON and DOM-1 on the expression of tight junction proteins in jejunum with Peyer patches.

Piglets were exposed by gavage for 21 days to 0.5 nmol/kg BW/day of DON (black bars), DOM-1 (grey bars) or kept as control animals (white bars).

Figure illustrate western blotting experiments: tissue was scraped, lysed, and 15 µg of proteins was analyzed by immunoblotting with antibodies for Claudin-4, Occludin, E-cadherin and β-actin as a protein loading control. Representative immunoblots and normalized expression graph.

Results are expressed as mean ± SEM of 6-8 independent experiments, p<0.05.

ESM_4 : Table of the effects of DON and DOM-1 exposure (0.5 nmol/Kg BW/day) on some biochemical values in plasma.Data are mean \pm SD for 6 to 8 animals. Means in a row with different letter differ ($p < 0.01$).

Biochemical parameters	D 7			D21		
	Control	DON	DOM-1	Control	DON	DOM-1
Alkaline phosphatase (U/L)	258.23 \pm 18.78 ^a	263.17 \pm 23.57 ^a	237.05 \pm 16.09 ^a	224.40 \pm 16.66 ^a	244.07 \pm 14.50 ^a	220.48 \pm 15.43 ^a
Alanine aminotransferase (U/L)	40.62 \pm 1.77 ^a	44 \pm 2.40 ^a	41.67 \pm 1.91 ^a	49.62 \pm 1.50 ^a	47.87 \pm 1.16 ^a	46.8 \pm 2.18 ^a
Aspartate aminotransferase (U/L)	49.38 \pm 3.35 ^a	57.62 \pm 4.66 ^a	51 \pm 6.20 ^a	46.5 \pm 8.03 ^a	38.75 \pm 2.67 ^a	44.2 \pm 3.29 ^a
Albumin (μ mol/L)	474.51 \pm 15.73 ^a	537.5 \pm 47.91 ^a	483.14 \pm 21.07 ^a	512.7 \pm 17.67 ^a	531.51 \pm 12.09 ^a	532.3 \pm 16.69 ^a
Total bilirubin (μ mol/L)	0.47 \pm 0.08 ^a	0.94 \pm 0.26 ^b	0.36 \pm 0.05 ^a	0.59 \pm 0.03 ^a	0.71 \pm 0.10 ^a	0.54 \pm 0.08 ^a
Cholesterol (mmol/L)	2.56 \pm 0.08 ^a	2.47 \pm 0.15 ^a	2.24 \pm 0.19 ^a	2.72 \pm 0.07 ^a	2.64 \pm 0.14 ^a	2.51 \pm 0.12 ^a
Total proteins (g/L)	49.75 \pm 0.91 ^a	49.4 \pm 0.64 ^a	47.98 \pm 1.46 ^a	53.86 \pm 1.15 ^a	53.96 \pm 1.66 ^a	55.83 \pm 1.76 ^a

ESM_5 : Table of total IgG, IgA or IgM (mg/ml) per treatment (control (white), DON (0.5 nmol/Kg BW, black) or DOM-1 (0.5 nmol/Kg BW, grey)), at D7, D14 and D21.

Values are mean \pm SEM for 6 to 8 animals. Means in a row with different letter differ ($p < 0.01$).

		Control	DON	DOM-1
IgG	<i>D7</i>	93 \pm 8 ^a	92 \pm 3 ^a	84 \pm 10 ^a
	<i>D14</i>	116 \pm 10 ^a	106 \pm 11 ^a	96 \pm 11 ^a
	<i>D21</i>	106 \pm 13 ^a	85 \pm 10 ^a	77 \pm 14 ^a
IgA	<i>D7</i>	156 \pm 16 ^v	117 \pm 15 ^a	186 \pm 37 ^a
	<i>D14</i>	187 \pm 27 ^a	176 \pm 16 ^a	212 \pm 27 ^a
	<i>D21</i>	194 \pm 34 ^a	186 \pm 15 ^a	308 \pm 67 ^a
IgM	<i>D7</i>	98 \pm 12 ^a	94 \pm 8 ^a	106 \pm 14 ^a
	<i>D14</i>	93 \pm 14 ^{a,b}	64 \pm 8 ^a	104 \pm 14 ^b
	<i>D21</i>	105 \pm 16 ^a	75 \pm 9 ^a	111 \pm 17 ^a