



Supplementary material

Deoxynivalenol, induces local inflammation and lesions in tissues at doses recommended by the EU

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Abstract: The mycotoxin deoxynivalenol (DON) is frequently present in cereals at low levels, resulting in its occurrence in food and feed. DON has been proven to alter the immune response and induce inflammation in all species, with pigs exhibiting heightened sensitivity and exposure. However, no study has yet evaluated the effects of exposure to DON at the recommended levels in pig feed. In two separate trials, piglets were subjected to control feed or feed contaminated with a low level of purified DON (0.83 mg/kg feed in trial 1 and 0.85 mg/kg feed in trial 2) for either three weeks (trial 1) or two weeks (trial 2). Additionally, a group of animals exposed to 2.85 mg/kg feed of DON was included as a positive control in Trial 1. The impact of DON on porcine tissues (intestine, liver, and spleen) was evaluated through histological and qPCR analyses of immune-related genes. Additionally, biochemical analyses and acute-phase proteins were examined in plasma samples. Lesions were identified in the intestine (jejunum and ileum), the liver, and the spleen of pigs receiving diets contaminated with low and high concentrations of DON. The low level of DON also resulted in impaired expression of genes associated with intestinal barrier integrity, intestinal immune responses, and liver function. In conclusion, the results of the two trials demonstrate the impact of DON exposure even at doses below the recommended level of 0.9 mg/kg feed set by the European Union. This suggests that the current recommended level should be reconsidered to ensure the optimal health and well-being of pigs.

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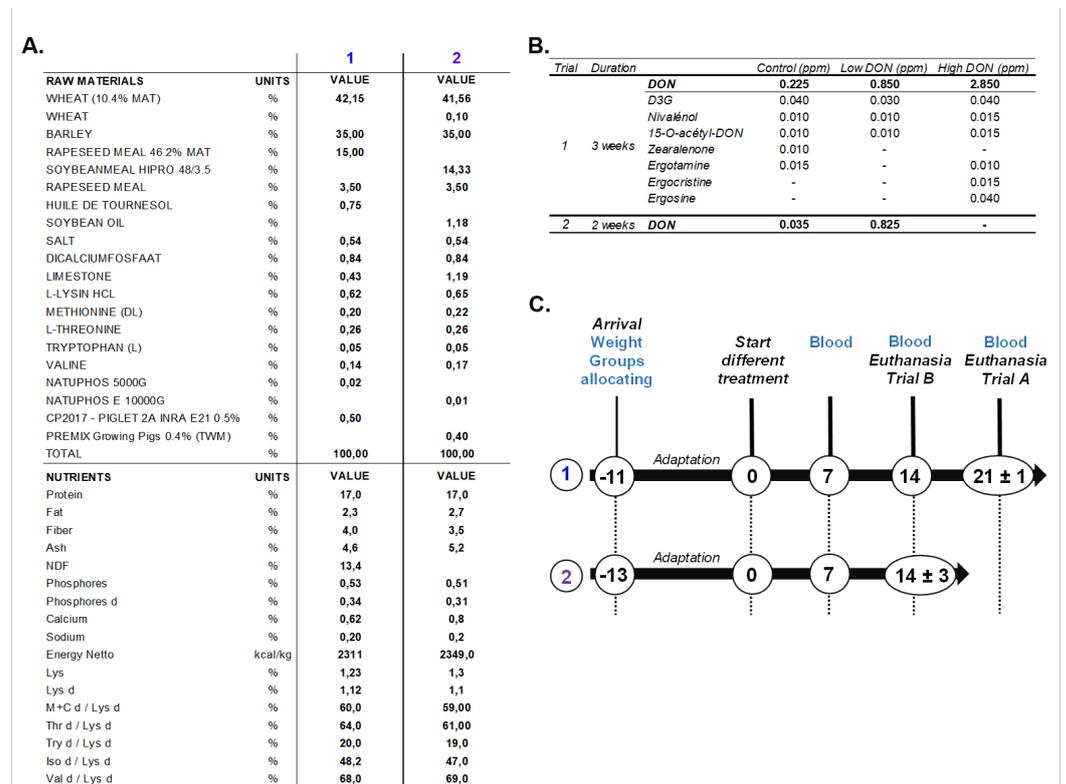


Figure S1: Experimental details of the animal trials. A) Animals were exposed to a second age formulated diet during all the experiment. A same diet formulation was calculated for the two trials but furnished by two different feed producers, inducing small changes. B) Real concentration of deoxynivalenol and some others mycotoxins or metabolites above their detection level obtained in the different regiment for the Trial 1 and the Trial 2. C) Experimental scheme for the Trial 1 and trial 2. for both trials, animals arrived at weaning age then passed by an adaptation phase of 11 or 13 days. Then were exposed to the different regiments during three weeks for the Trial 1 and two weeks for the Trial 2. Every week, animals were weighed and their blood was collected. At the end of the experiment they were euthanized and different organs were collected.

Table S1: Primer sequences used for RT-qPCR analysis (F: Forward, R: reverse).

Gene Symbol	Gene Name	Primer sequence	References
HMBS	Hydroxymethylglutaryl synthase	F: AGGATGGGCAACTCTACCTG R: GATGGTGGCCTGCATAGTCT	ENSSSCT0000060506.2 Pierron et al. 2022
B2M	Beta-2-Microglobulin	F: CTGCTCTCACTGTCTGG R: TTCAGGTAATTTGGCTTCC	ENSSSCT00005043116.1 Pierron et al. 2022
RPL32	Ribosomal protein	F: AGTTCATCCGGCACCAGTCA R: GAACCTTCTCCGCACCCTGT	NM_001001636 Pinto, et al. 2010
TOP2B	DNA Topoisomerase II Beta	F: AAGGGCGAGAGGTCAATGAT R: ACATCTTCTCGTTCTTGCGC	AY550069 Park et al. 2015
IL1 beta	Interleukin 1 beta	F: ATGCTGAAGGCTCCACCTC R: TTGTTGCTATCATCTCCTTGAC	NM_214055 Braccarone et al. 2020
IL1 alpha	Interleukin 1 alpha	F: GCCAATGACACAGAAGAAGA R: ATGCACTGGTGGTTGATG	NM_214029 Pierron et al. 2022
CXCL8	Interleukine 8	F: CTTCTGTGAGGCTGCAGTTC R: AAGGTGTGGAATGCGTATTTATGC	NM_213867 Cano et al. 2013
IL10	Interleukine 10	F: GGCCCAAGTGAAGAGTTCTTTC R: CAACAAGTCGCCCATCTGGT	NM_214041 Cano et al. 2013
IFNgamma	Interferon gamma	F: TGGTAGCTCTGGGAAACTGAATG R: GGCTTTCGCTGGATCTG	NM_213948 Cano et al. 2013
TNFalpha	Tumor necrosis factor alpha	F: ACTGCACCTCGAGTTATCGG R: GCGCAGGGCTTATCTGA	NM_214022 Cano et al. 2013
IL12p40	Interleukin 12 subunit 40	F: GGTTTCAGACCCGACGAACCTC R: CATATGGCCCAATGGGAGATG	NM_214013 Cano et al. 2013
TGFbeta1	Transforming Growth Factor 1	F: GGATACCACTACTGCTTCAG R: GGTTTCATGAATCCACTCCA	ENSSSCT0000036469.2 This Study
NFKB1	Nuclear Factor Kappa B	F: CCTCCACAAGGCAGCAAAATAG R: TCCACACCGCTGCACAGA	ENSSSCT0000033438 This Study
IGFB1	Insulin-like Growth Factor 1	F: TTCAGTTCGTGCGGAGAC R: CGTACCTGTGGCTTGTG	NM_214256.1 This Study
MUC2	Mucin 2	F: GCAGCCTGTGCGAGGAA R: TGTCATCATACAGTGCCTTCTG	XM_003122394.1 Pinto, et al. 2015
CCK	Cholecystokinin	F: AAAGCACCTTCTGGCCGAGT R: GGTCATTATTCTGTGGCTGGG	NM_214237 This Study
SOD1	Superoxyde Dismutase 1	F: ATCATGGATTCCATGCCATCAG R: GGACCTGCACCTGGTACAGCC	NM_001190422 This Study
SOD2	Superoxyde Dismutase 2	F: AGTACCAGGAGGCGCTGAAA R: GCAGAGCTACCTGAGCTGAACATC	NM_214127 This Study
ABCA1	ATP-binding cassette, sub-family A member 1	F: ACCTGGTGAAGACCTATGTGCAG R: CAGATCTTGTCTTTAAGCTTTGGC	ENSSSCT0000027427 This Study
Casp3	Procaspase 3	F: ATAATAAGAACTTTGATAAAAAACCGGAATG R: TCCACATCTGTACCAGATCGACAT	NM_214131 This Study
Casp9	Caspase 9	F: CACACCCAGTGACATCTTT R: CAAAGCGACTGGATGAAC	AB020979.1 Hasuda et al. 2022

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References

- Hasuda, A.L.; Person, E.; Khoshal, A.K.; Bruel, S.; Puel, S.; Oswald, I.P.; Bracarense, A.P.F.R.L.; Pinton, P. Deoxynivalenol induces apoptosis and inflammation in the liver: Analysis using precision-cut liver slices. *Food Chem. Toxicol.* **2022**, *163*, 112930.
- Bracarense, A.P.F.L.; Pierron, A.; Pinton, P.; Gerez, J.R.; Schatzmayr, G.; Moll, W.D.; Zhou, T.; Oswald, I. P. Reduced toxicity of 3-epi-deoxynivalenol and de-epoxy-deoxynivalenol through deoxynivalenol bacterial biotransformation: In vivo analysis in piglets. *Food Chem. Toxicol.* **2020**, *140*, 111241.
- Pierron, A.; Neves, M.; Puel, S.; Lippi, Y.; Soler, L.; Miller, J. D.; Oswald, I.P. Intestinal toxicity of the new type A trichothecenes, NX and 3ANX. *Chemosphere.* **2022**, *288*, 132415.
- Pinton P, Braicu C, Nougayrede JP, Laffitte J, Taranu I, Oswald IP. Deoxynivalenol impairs porcine intestinal barrier function and decreases the protein expression of claudin-4 through a mitogen-activated protein kinase-dependent mechanism. *J Nutr.* **2010**, *140*, 1956-62.
- Park, S.J.; Kwon, S.G.; Hwang, J.H.; Park, D.H.; Kim, T.W.; Kim, C.W. Selection of appropriate reference genes for RT-qPCR analysis in Berkshire, Duroc, Landrace, and Yorkshire pigs. *Gene.* **2015**, *558*, 152–158.
- 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.
- Pinton, P.; Graziani, F.; Pujol, A.; Nicoletti, C.; Paris, O.; Ernouf, P.; Di Pasquale, E.; Perrier, J.; Oswald, I.P.; Maresca, M. Deoxynivalenol inhibits the expression by goblet cells of intestinal mucins through a PKR and MAP kinase dependent repression of the resistin-like molecule β . *Mol. Nutr. Food Res.* **2015**, *59*, 1076-87.

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