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Deoxynivalenol alone or in combination with nivalenol and zearalenone induce systemic histological changes in pigs

Juliana R. Gerez [a], Philippe Pinton [b,c], Patrick Callu [d], François Grosjean [d], Isabelle P. Oswald [b,c], Ana Paula F.L. Bracarense [a,*]

[a] Universidade Estadual de Londrina, Laboratory of Animal Pathology, Londrina, Brazil
[b] INRA, Toxalim UMR 1331, Research Center in Food Toxicology, F31027 Toulouse, France
[c] University of Toulouse, INP, ToxAlim, UMR 1331, F31027 Toulouse, France
[d] Arvalis Institut du Végétal, Pouline, 41100 Villerable, France

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ABSTRACT

Deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) are mycotoxins commonly produced by *Fusarium* species. The purpose of the present study was to investigate the effects of DON alone and in combination with NIV and ZEA on several parameters including weight gain and histological aspects of pigs submitted to chronic intoxication. Twenty, 5-week-old piglets received for 28 days one of the following diets: a control diet, a diet mono- contaminated with DON (1.5 mg/kg), a diet multi-contaminated with DON (2 mg/kg) + NIV (1.3 mg/kg) + ZEA (1.5 mg/kg) or a diet contaminated with DON (3 mg/kg) + NIV (1.3 mg/kg) + ZEA (1.5 mg/kg). Animals fed the multi-contaminated diets presented a significant decrease in weight gain over the total period. The chronic ingestion of the contaminated diets induced a significant increase on histological changes on the intestine, liver and lymphoid organs. In addition, a significant increase on lymphocyte apoptosis was observed in lymph nodes and spleen in the animals receiving the contaminated diets. These data provide a better understanding of the possible effects of *Fusarium* toxins, alone or in combinations on the morphology of the intestine and lymphoid organs, which would contribute to the risk assessment of these toxins.

1. Introduction

Mycotoxins are secondary metabolites produced by a wide variety of fungal species that cause nutritional losses and represent a significant hazard to the food chain. According to the Food and Agriculture Organization (FAO) of the United Nations, it is estimated that approximately 25% of the cereals produced in the world are contaminated by mycotoxins (Rice and Ross, 1994).

Zearalenone (ZEA), fumonisin B1 (FB1) and trichothecenes, in particular, deoxynivalenol (DON) and nivalenol (NIV) are amongst the most toxicologically important *Fusarium* toxins that occur frequently in combination in cereal grains. In Europe, DON is the major contaminant, often in co-occurrence with ZEA (Streit et al., 2012). However, to date, very little is known about the potential interactive toxic effects among fusariotoxins (Grenier and Oswald, 2011; Wan et al., 2013a).

It is well-known that trichothecenes induce various toxic effects in animals such as suppression of body growth and immune function, diarrhea, and general loss of condition. Trichothecenes exhibit inhibitory influence on protein synthesis by binding to ribosomes, and inhibition of DNA and RNA synthesis has also been reported (Hascheck et al., 2002). Therefore, organs/tissues showing high rate of cell turnover are regarded as particularly susceptible to trichothecenes, such as the lymphoid and hematopoietic tissues, and the gastrointestinal tract (Hascheck et al., 2002; Rocha et al., 2005). In addition, trichothecenes have been shown to affect immunological functions by deregulating production of cytokines and immunoglobulins and by inducing apoptosis (Bondy and Pestka, 2000; Pestka et al., 2004).

DON causes toxic and immunotoxic effects in a variety of cell systems and animal species (Hascheck et al., 2002; Pestka and Smolinski, 2005). Swine are more sensitive to DON than other species, in part because of differences in the metabolism of DON. Chronic low dietary concentrations (0.28; 0.56; 0.84 mg/kg of feed) induced no changes on animal performance, biochemical and hematological variables and immunological parameters (Accensi et al., 2006), while acute higher doses induce vomiting,

* Corresponding author. Tel.: +55 43 33714485; fax: +55 43 33714485.
E-mail addresses: anapaula@uel.br, ana.bracarense@pq.cnpq.br (A.P.F.L. Bracarense).

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Table 1
Composition of the pigs experimental diets.

Ingredients (%)	Control	DON	DON[1] + NIV + ZEA	DON[2] + NIV + ZEA
Wheat	75.41	5.37	52.43	0
Contaminated wheat	0	70.00	70.00	53.77
Contaminated maize andoins	0	0	13.00	13.00
Contaminated maize cribs	0	0	8.50	8.00
Soybean	19.5	18.5	21.00	19.6
Lysine HCL	0.61	0.64	0.58	0.63
Threonine	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20
Tryptophan	0.03	0.04	0.04	0.05
Vitamin and mineral premix	4.00	4.00	4.00	4.00
Vegetable oil	0	1.00	0	0.50
Composition (%)				
Crude protein	20.43	20.48	20.33	20.20
Crude fiber	2.75	3.08	2.77	3.02
Ethereus extract	1.29	2.70	1.83	2.61
Digestible energy	3866	3851	3861	3833
Net energy	2770	2771	2775	2764
Lysine D	1.34	1.35	1.33	1.34
Ca	1.46	1.46	1.53	1.53
P	0.80	0.79	0.79	0.69
Mycotoxin (mg/kg)				
DON	0.12	1.5	2.08	3.0
NIV	<LQ(0.2)	<LQ(0.2)	1.3	1.3
ZEA	0.02	0.18	1.5	1.5
Fusarenon X	<LQ(0.01)	<LQ(0.01)	<LQ(0.01)	<LQ(0.01)
T-2 toxin	<LQ(0.01)	<LQ(0.01)	0.01	0.012
T-2 toxin	<LQ(0.01)	<LQ(0.01)	0.025	0.020
Diacetoxyscispernol	<LQ(0.05)	<LQ(0.05)	<LQ(0.05)	<LQ(0.05)
3 + 15 acetyl DON	<LQ(0.01)	<LQ(0.01)	<LQ(0.01)	<LQ(0.01)
Fumonisin B1	<LQ(0.01)	<LQ(0.01)	0.21	0.20
Fumonisin B2	<LQ(0.01)	<LQ(0.01)	<LQ(0.01)	<LQ(0.01)

DON[1] (2.0 mg/kg) + NIV (1.3 mg/kg) + ZEA (1.5 mg/kg); DON[2] (3.0 mg/kg) + NIV (1.3 mg/kg) + ZEA (1.5 mg/kg).

hemorrhagic diarrhea and circulatory shock (Pestka and Smolinski, 2005).

Long-term NIV chronic exposure in mice induced a reduced body gain and feed efficiency, and an increase in relative organ weight or severe leucopenia (Ryu et al., 1988). However, young pigs exposed to NIV by three weeks showed no changes in body or organ weight, but in the macroscopic examination revealed lesions in the kidneys and gastrointestinal tract and reduction in the number of splenocytes (Hedman et al., 1997). Associated with other trichothecenes, NIV has been shown to correlate with the high incidence of esophageal cancer in China (Hsia et al., 2004).

ZEA and its metabolites exhibit potent estrogenic activity; hence it is often referred as a mycoestrogen. This non-steroidal mycostrogen binds to estrogen receptors leading to hyperestrogenicity in several animal species, especially pigs (Minervini and Dell'Aquila, 2008). *In vivo* studies showed that rats fed with ZEA developed liver lesions and hepatocarcinomas (NTP, 1982). Prepuberal gilts fed diets contaminated with DON (2.1 to 9.57 mg/kg) and ZEA (0.004 to 0.358 mg/kg) showed hepatocyte glycogen depletion and expansion of hepatic interlobular connective tissue (Tiemann et al., 2006a) and hemossiderosis in spleen (Tiemann et al., 2006b). ZEA induced intracellular oxidative stress that results in induction of oxidative DNA damage (Hassen et al., 2007) and apoptosis (Abid-Essefi et al., 2004). The immune system is a potent target for estrogenic endocrine disruptors considering that its cells express estrogenic receptors (Igarashi et al., 2001). In spite of that, only few studies have been carried out on the immune effects of ZEA and its metabolites. In particular, reduction of mitotic index and cell survival of porcine lymphocytes was reported when high concentrations of ZEA were used in *in vitro* (Luongo et al., 2008). ZEA and its derivatives showed toxic effects on porcine neutrophils and decreased IgG, IgA and IgM levels as well as TNF-α synthesis in an *in vitro* model (Marin et al., 2010, 2011).

Considering that food and feed commodities are often contaminated by more than one mycotoxin (Speijers and Speijers, 2004), studying the interactions between different mycotoxins can be useful. It is known that *Fusarium* toxins can exert additive and synergistic effects (Tajima et al., 2002), but mycotoxins may also act as antagonists. The data on combined toxic effects of mycotoxins are limited and, therefore, the actual combined health risk from exposure to mycotoxins is unknown (Grenier et al., 2011). Assessment of the interaction of *Fusarium* mycotoxins has been investigated *in vitro* on porcine immune cells, swine and human intestinal epithelial cells (Luongo et al., 2008; Kouadio et al., 2007; Wan et al., 2013a,b). *In vivo* experiments have also been performed on mice, pigs and poultry using high doses of toxins in which the authors mainly looked for differences in animal performance (Grenier et al., 2011). Among them, few studies were concerned with the interaction between the *Fusarium* toxins DON, NIV and ZEA (Luongo et al., 2008).

The purpose of this study was to evaluate the weight gain and to determine the extent of histological lesions in selected organs of piglets submitted to chronic intoxication by ingestion of feed naturally contaminated with DON or multi-contaminated with DON, NIV and ZEA.

2. Material and methods

2.1. Animals and diet

The experiment was conducted in Arvalis–Institut du vegetal (Villerable, France) facilities. Twenty castrated male piglets (Pietrain), 5-week-old were divided in four groups of homogeneous