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Histological structure of duodenum in gilts receiving low doses of zearalenone and deoxynivalenol in feed

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ABSTRACT

Deoxynivalenol (DON) and zearalenone (ZEN), produced by microfungi of the *Fusarium* family, are among the most commonly occurring mycotoxins. They are considered important factors affecting human and animal health as well as livestock productivity. The aim of this study was to determine the effect of low doses of these mycotoxins on the histological structure of the pig duodenum. The study was performed on 72 gilts, with initial weights of approximately 25 kg, divided into 4 equal groups. Group I received *per os* ZEN (40 μg/kg BW), group II—DON (12 μg/kg BW), group III—ZEN (40 μg/kg BW) and DON (12 μg/kg BW), and group IV—vehicle. The pigs were killed after 1, 2, 3, 4, 5 and 6 weeks of the treatment, and the duodenum samples were prepared for histological investigations. The slides were digitalized and subjected to morphometrical analysis. The treatment with DON and ZEN did not change the architecture of the mucosa or the ratio between goblet and adsorptive cells in the epithelium. The administration of DON induced an increase in the number of lymphocytes in the mucosal epithelium. Both mycotoxins, administered alone or together, increased the quantity of lymphocytes, plasma cells and macrophages with black-brown granules in the lamina propria. The time-courses of changes in the number of defense system cells evoked by DON and ZEN were different. In conclusion, dietary exposure to low doses of *Fusarium* mycotoxins should be considered an important risk factor for subclinical inflammation in the small intestine.

1. Introduction

Mycotoxins produced by various fungi species are commonly occurring contaminants of food and feed ingredients. It is estimated that 25% of the world crop production contains mycotoxins (Bullerman, 1996; Shephard et al., 1996). Their consumption is considered a serious health hazard for both humans and animals (Canady et al., 2001). Mycotoxins may be present in a diet at high concentrations, causing prominent adverse effects (Bhat et al., 1989); however, in the majority of cases, their levels are too low to induce immediate clinical symptoms. Despite the lack of directly occurring manifestations of disease, the intake of low doses of mycotoxins may result in more or less serious damage to cells, tissues and organs as well as promote the development of neoplasmatic processes (Canady et al., 2001). The absence of warning symptoms frequently leads to long-term consumption of contaminated food or feed. The chronic exposure of farm animals to mycotoxins usually results in decreased growth rate (Côté et al., 1984, 1985; Dänicke et al., 2005a; Döll and Dänicke, 2011; Andretta et al., 2012; Flannery et al., 2012), reproductive disturbances (Zwierzchowski et al., 2005; Döll and Dänicke, 2011) and increased occurrence of various non-infectious and infectious diseases, the latter mainly due to suppression of the defense mechanisms (Bouhet et al., 2004; Pestka, 2007; Pinton et al., 2008; Döll and Dänicke, 2011; Vandenbroucke et al., 2011). Mycotoxins cause significant economic losses to farm animal industries (Iheshiulor et al., 2011; Andretta et al., 2012). More serious health effects of the long-term consumption of mycotoxins in a diet, including severe gastrointestinal tract diseases and malignant tumors, are observed in humans and accompanying animals, most likely due to longer lifespan (Wild and Gong, 2010; Cano et al., 2013).

Deoxynivalenol (DON) and zearalenone (ZEN), secondary metabolites of *Fusarium* fungi (*Fusarium graminearum, Fusarium culmorum* and *Fusarium roseum*), are among the most commonly occurring mycotoxins. They are formed in fields prior to harvest,

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and their occurrence cannot be completely avoided by production-minimizing strategies due to the major impact of weather conditions as well as the high chemical and thermal resistance of these compounds.

DON, also called vomitoxin, acts at a cellular level as a protein synthesis inhibitor (Dänicke et al., 2006; He et al., 2012) and a stimulator of mitogen-activated protein kinases (Pestka, 2007, 2010; Lucioli et al., 2013); therefore, DON can cause a wide variety of biological effects (Lucioli et al., 2013; Pinton and Oswald, 2014; Pinton et al., 2009, 2012, 2015). It has been demonstrated that DON alters the expression of claudin and occludin in the epithelial cells and damages the intestinal barrier, increasing its permeability to bacteria (Pinton et al., 2009; Bracarense et al., 2012; Pinton and Oswald, 2014). DON also affects the composition of the basement membrane proteins and influences the route of lymphocyte migration into the intestinal epithelium (Nossol et al., 2013). Moreover, the mycotoxin induces a proinflammatory response through stimulation of cytokines formation (Pestka, 2007, 2010; Pinton and Oswald, 2014). Cytokines seem to be responsible for several negative effects of DON. Anorexia induced by DON is a consequence of changes in various regulatory mechanisms (Pinton and Oswald, 2014), including the secretion of serotonin and gut peptide YY (Flannery et al., 2012). Exposure to high doses of DON causes acute gastroenteritis with diarrhea and vomiting, which pathogenesis may involve damage of intestinal epithelial cells, as well as dysregulations of the immune and neuroendocrine systems (Côté et al., 1985; Dänicke et al., 2005a; Pestka, 2007; Döll and Dänicke, 2011). The chronic effects of low doses involve anorexia, nutrient malabsorption, reduced weight gain as well as neuroendocrine and immunological changes (Pinton et al., 2008; Flannery et al., 2012; Bracarense et al., 2012).

ZEN and its metabolites possess an estrogen-like activity and compete with the endogenous hormones for the binding sites of estrogen receptors (Gajęcki, 2002; Döll and Dänicke, 2011). Moreover, ZEN interferes in the steroid metabolism by altering the activities of enzymes: 3-β-hydroxysteroid dehydrogenase type 1, cytochrome P450 side-chain cleavage enzyme, and P450 aromatase, as well as by influencing steroidogenic acute regulatory protein (Döll and Dänicke, 2011). The administration of ZEN results in hyperestrogenism, precocious puberty and reproductive disorders (Gajęcki, 2002; Döll and Dänicke, 2011). Exposure of sows to ZEN during pregnancy and lactation reduces the quantity of healthy follicles in piglets of the F1 generation (Schoevers et al., 2012). ZEN and its metabolites also have negative effects on immune functions (Marin et al., 2011, 2015).

In many cases, a diet contains DON and ZEN simultaneously; however, the interactions between the two mycotoxins are almost unknown and very difficult to predict (Döll and Dänicke, 2011; Pinton and Oswald, 2014).

The digestive system, as the main location of resorption and metabolism of mycotoxins, is strongly influenced by these dangerous compounds (Avantaggiato et al., 2004; Dänicke et al., 2005b; Cavret and Lecoeur, 2006; Dong et al., 2010). The tunica mucosa of the gastrointestinal tract is exposed to much higher concentrations of the ingested mycotoxins than any other structure of the body. Therefore, special attention should be paid to the study of the effects of these compounds on the function and structure of the gastrointestinal tract.

Among farm animals, the pig is the most sensitive species to both DON and ZEN, reacting to low doses of DON with a decrease in voluntary feed intake and a reduction in growth rate (Andretta et al., 2012), and to low doses of ZEN with fertility disorders (Gajęcki, 2002; Pestka, 2007; Döll and Dänicke, 2011). Thus, both mycotoxins are responsible for important economic losses in the pig farm industry (Andretta et al., 2012).

The aim of this study was to determine the effects of low doses of DON and ZEN, administered solely or jointly for 1–6 weeks, on the histological structure of the duodenum in the pig. This study is the first to address the influence of *Fusarium* mycotoxins on the duodenum of mammals.

2. Material and methods

2.1. Animals and experimental procedures

The study was performed on 72 clinically healthy gilts of mixed breed (White Polish Big × Polish White Earhanging), weighing 25 ± 2 kg at the beginning of the experiment. The pigs were purchased from a farm where they received feed free from detectable amounts of ZEN, DON, α-zearalenol, aflatoxin, and ochratoxin. Serological tests were carried out to exclude Auyeski's disease, mycoplasmosis, parvovirosis, actinobacillosis and porcine reproductive-respiratory syndrome in these animals. The tests for internal and external parasites also gave negative results.

The pigs were randomly divided into four equal groups and acclimatised for 2 weeks in the animal facility of Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn. Then, group I received ZEN in a dose of 40 μg/kg BW, group II—DON in a dose of 12 μg/kg BW, and group III—ZEN in a dose of 40 μg/kg BW and DON in a dose of 12 μg/kg BW. Mycotoxins were synthetized, purified and standardized at the Department of Chemistry, Faculty of Wood Technology, Poznań University of Life Sciences, Poland. The gilts were weighed every week to establish the amount of DON and ZEN given to each animal. The mycotoxins were administered orally during the morning feeding in water-soluble capsules containing oat bran as the vehicle. Based on the animal weight and average feed intake, it could be estimated that the doses of mycotoxins correspond to concentrations of 250 μg DON and 833 μg ZEN per 1 kg of feed. Group IV served as a control and received capsules without mycotoxins. The pigs were killed by intravenous administration of sodium pentobarbital (Vetbutal, Biowet, Poland) and exsanguination after 1–6 weeks of the experiment.

All procedures were conducted in compliance with Polish legal regulations determining the terms and methods for performing experiments on animals and the European Community Directive for the ethical use of experimental animals. The protocol was approved by the Local Ethical Council in Olsztyn (opinion No. 88/N of 16 December 2009).

2.1.1. Histological studies

The tissue samples (approximately 1 cm × 0.5 cm) were cut from the initial and middle parts of the duodenum immediately after the heart stopped beating. They were flushed with saline and fixed in 4% paraformaldehyde solution in 0.1 M phosphate buffer (pH 7.4) for 48 h, dehydrated in ethanol (TP 1020, Leica, Germany) and embedded in paraffin (EG1150, Leica, Germany). Then, 4-μm-thick sections, prepared with the use of a HM 340E microtome (Microm, Spain), were stained by the hematoxylin & eosin method (HE), the periodic acid Schiff method (PAS) or the methyl green-pyronine staining (MGP) using a ST 5020 multistainer (Leica, Germany). Additionally, some sections were processed with Prussian blue staining for the detection of iron. The slides were scanned using a MiraxDesk scanner (Carl Zeiss, Germany) and analyzed using the Pannoramic Viewer 1.12 software (3D-Histech, Hungary). In addition, some sections were investigated using an Axioimager optic microscope equipped with an AxioCam MRc5 digital camera (Carl Zeiss, Germany). The slides were identified in a way that obscured the type and duration of the animal treatment from the people performing the microscopic analysis.