Original article

Effect of deoxynivalenol on the levels of toll-like receptors 2 and 9 and their mRNA expression in enterocytes in the porcine large intestine: a preliminary study

M. Dąbrowski, E. Jakimiuk, M. Gajęcka, M.T. Gajęcki, Ł. Zielonka

Department of Veterinary Prevention and Feed Hygiene, Faculty of Veterinary Medicine,
University of Warmia and Mazury, Oczapowskiego 13, 10-718 Olsztyn, Poland

Abstract

Deoxynivalenol (DON), one of the most prevalent mycotoxins in the world, and is capable of inducing immune disorders in humans and animals. The aim of this study was to determine the effect of feed contaminated with DON on the number of TLR2- and TLR9-positive cells and their mRNA expression in the porcine large intestine. The experiment was conducted on two equal groups of pigs (n=4). The experimental group (E) was administered feed contaminated with DON (1008 μg/kg of feed) for 6 weeks, and the control group (C) was administered non-contaminated feed over the same period of time. A decrease in the expression of TLR2 mRNA was noted in the cecum. The percentage of TLR9-positive enterocytes increased in the ascending colon and decreased in the cecum. The results of this study indicate that DON can modify the local immune response by changing the expression of TLRs.

Key words: deoxynivalenol, toll-like receptors 2 and 9, large intestine, porcine

Introduction

Mycotoxins are secondary fungal metabolites that contaminate cereals and cereal products (Abysique et al. 2015). Deoxynivalenol is the most common group B trichothecene in cereals such as wheat, maize, barley, oats, rye, and it is less frequently isolated from rice and bananas (Binder 2007). Rodrigues and Naehrer (2012) analyzed 7049 cereal and feed samples in 2009-2011 and identified DON in 59% of the samples. The global prevalence of mycotoxins poses a significant risk to human and animal health (Fink-Gremmels 1999).

In humans and animals, high doses of DON provoke nausea, vomiting, sleepiness, diarrhea, leukocytosis, gastrointestinal bleeding and, in extreme cases, death (Bryden 2007). Exposure to moderate concentrations of this mycotoxin leads to anorexia, lower weight gains and lower feed efficiency (Pestka and Smolinski 2005). Similarly to other trichothecenes, DON exhibits tropism for rapidly proliferating and differentiating cells and tissues, in par-

ticular in the intestines and the immune system (Feinberg and McLaughlin 1989). Contaminated food or feed exposes the intestinal epithelium to high concentrations of potentially harmful mycotoxins (Bouhet and Oswald 2005). In the gastrointestinal tract, DON provokes shortening of intestinal villi, lysis and swelling of enterocytes (Kolf-Clauw et al. 2009). Caco-2 cells chronically exposed to DON induce the phosphorylation of mitogen-activated protein kinases (MAPKs) and lower transepithelial electrical resistance (TEER) (Sergent et al. 2006).

Trichothecenes target ribosomes at the molecular level. Those mycotoxins bind to the eukaryotic 60S ribosomal subunit (Middlebrook and Leatherman 1989). The above initiates a process known as the ribotoxic stress response which inhibits protein synthesis and activates MAPKs (Iordanov et al. 1997). After binding with ribosomes, DON inhibits ribosomal peptidyl transferase, which prevents the initiation and elongation of the translation process (Shifrin and Anderson 1999). The inhibition of protein synthesis induced by DON can also result from the degradation of 28S rRNA (Li and Pestka 2008). Zhou et al. (2003) demonstrated that DON can activate double-stranded RNA-associated protein kinase (PKR) which is responsible for the phosphorylation of the eukaryotic initiation factor 2α (eIF2α) and inhibition of translation. He and Pestka (2010) reported that DON can bind with mRNA to activate microRNA molecules responsible for selective gene silencing and inhibiting protein synthesis.

Deoxynivalenol influences the transcription of genes encoding cytokines and chemokines by inducing and activating transcription factors such as NF-kB, CREB and AP-1 (Pestka 2010). It also stabilizes mRNA molecules and increases their concentrations inside cells (Chung et al. 2003).

Toll-like receptors act as pathogen recognition receptors (PRRs) in mammals where they are responsible for the recognition of pathogen-associated molecular patterns (PAMPs) (Akira and Takeda 2004). In pigs, TLRs are the first line of defense against pathogens, and their ligands are used as vaccine adjuvants (Uenishi and Shinkai 2009). The current state of knowledge indicates that TLRs and their signaling molecules play a key role in regulating gut inflammatory processes caused by invasive pathogens (Burkey et al. 2009). Toll-like receptor 2 plays a role in the recognition of bacterial cell wall components: peptidoglycan, lipoteichoic acid, lipoproteins, lipoarabinomannans, mycobacteria and zymosan (Wetzler 2003). By binding with ligands, TLR2 induces the secretion of TNF-α, IL-2, IL6 and IL-12 (Thoma-Uszynski et al. 2000). Toll-like receptor 9 is involved in the recognition of unmethylated CpG-dense sequences which are commonly found in the DNA of viruses, bacteria and their synthetic homologs (oligodeoxynucleotides). It is also present inside cells in endosomal compartments (Latz et al. 2004).

The objective of this study was to determine the influence of feed contaminated with DON at levels similar to the reference doses recommended by the European Commission (EC 2006) on the expression of TLR2 and TLR9 mRNA and the percentage of TLR2- and TLR9-positive enterocytes in the porcine cecum, ascending and descending colon. The human digestive system and the porcine gastrointestinal tract are characterized by significant anatomical and immunological similarities; therefore, the results of an experiment performed on a porcine model can be extrapolated to humans (Patterson et al. 2008, Meurens et al. 2012).

Materials and Methods

All procedures relating to animal handling and collection of samples for analysis were carried out with the consent of the Local Ethics Committee in Olsztyn, decision No. 45/2013 of 19 September 2013.

Animals

The experiment was performed on 8 crossbred (Polish Large White x Polish Landrace) weaners with an estimated initial body weight of 23 kg. The animals were randomly divided into two equal groups: the experimental group (E) (n=4) administered contaminated feed and the control group (C) (n=4) whose feed was not contaminated with mycotoxins. The groups were kept in separate pens with free access to water. Feeding times were restricted to 8 a.m. and 5 p.m. The experiment was conducted for 6 weeks, after which the animals were euthanized by intravenous administration of pentobarbital sodium (Fatro, Ozzano Emilia BO, Italy) and bleeding. Sections of the cecum, ascending and descending colon were collected immediately after cardiac arrest and were prepared for analyses.

Immunohistochemical evaluation of TLR2 and TLR9 expression

Tissue sections were fixed in 4% paraformaldehyde solution in phosphate buffer and embedded in paraffin. Two specimens for every analyzed segment of the large intestine were prepared and stained to

detect the presence of TLR2 and TLR9, respectively. Serial microtome sections with a thickness of 5 μm were placed on glass slides (Menzel-Glaser, Braunschweig, Germany) and deparaffinized in xylene (Polskie Odczynniki Chemiczne SA, Gliwice, Poland) and a series of graded ethanol solutions. Tissue sections were placed in citrate buffer (Sigma-Aldrich, Saint Louis, MO, USA) and microwaved for 20 minutes at 800 W to unmask antigens. The sections were coated with DAKO REAL™ Peroxidase Blocking Solution (DAKO, Glostrup, Denmark) and incubated for 15 minutes to block endogenous peroxidase activity. Non-specific antibody binding sites were blocked with 2.5% normal horse serum. The prepared sections were incubated overnight at a temperature of 4ºC with primary antibodies: anti-porcine TLR9 (THU-A-TLR9 Cosmo Bio, Tokyo, Japan) and anti-porcine TLR2 (THU-A-TLR2 Cosmo Bio, Tokyo, Japan) diluted 1:200 and 1:300, respectively. After incubation, the specimens were rinsed three times in PBS (Sigma-Aldrich, Saint Louis, MO, USA) at 5 minute intervals. They were coated with secondary antibodies conjugated with horseradish peroxidase-labeled micro polymer (ImmPRESS™ HRP Universal Antibody, Vector Laboratories, Burlingame, CA, USA) and stained by incubation with DAB for 3 minutes (DAKO, Glostrup, Denmark). The resulting specimens were rinsed in water and counterstained with Mayer's hematoxylin (Sigma-Aldrich, Saint Louis, MO, USA). They were dehydrated in ethanol and xylene and mounted in DPX (Sigma-Aldrich, Saint Louis, MO, USA).

The preparations were scanned in a Pannoramic MIDI digital slide scanner (3DHISTECH Ltd., Budapest, Hungary). The expression of TLR2 and TLR9 was evaluated in a Panoramic Viewer (3DHISTECH Ltd., Budapest, Hungary), a dedicated application for virtual microscopy. The percentage of enterocytes positive for TLR2 and TLR9 was determined in 8 randomly selected fields of view at 40x magnification. The results were expressed by the mean percentage of cells positive for TLR2 and TLR9.

Analysis of TLR2 and TLR9 mRNA expression

Total RNA was isolated from mechanically homogenized tissues immediately after collection, with the use of the GeneJET RNA Purification Kit (Thermo Scientific, Waltham, MA, USA), according to the manufacturer's instructions. The quantity and quality of isolated RNA was checked spectrophotometrically (BioSpectrometer, Eppendorf, Hamburg, Germany). Isolated RNA (1 μg) was converted into cDNA using the Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Scientific, Waltham, MA, USA). The results of gene expression were normalized relative to the reference gene (β-actin). To compare the expression of the analyzed genes, the reaction was optimized to produce equivalent efficiencies. Comparable efficiency was validated by performing serial dilutions of the obtained cDNA and determining the threshold cycles (Ct) for every dilution. The slope of the obtained curve (representing cDNA concentration vs. Ct) was used to calculate reaction efficiency based on the formula $E = 10^{(-1/ curve\ slope)}$. Real-time PCR was carried out in a Rotor Gene 6000 system (Qiagen, Venlo, Netherlands) with a Luminaris Color HiGreen qPCR Master Mix (Thermo Scientific, Waltham, MA, USA) and specific primers: swine TLR2, TLR2-S: ACATGAAGATGATGTGGGCC (sense) and TLR2-A: TAGGAGTCCTGCTCACTGTA (antisense); swine TLR9, TLR9-S: GTGGAACTGTTTTGGCATC (sense) and TLR9-A: CACAGCACTCTGAGCTTTGT (antisense); swine β-actin (housekeeping gene), B-ACTIN-S: TGGCATTGTCATGGACTCTG (sense) and B-ACTIN-A: AGGGGCGATGATCTTGATCT (antisense) (Tohno et al. 2005, Shimosato et al. 2005). The following protocol was used: incubation with uracil-DNA glycosylase (UDG) at 50C for 2 minutes and initial denaturation at 95C for 10 s. This was followed by 40 amplification cycles with the following sequence: denaturation at 95C for 15 s, annealing at 57C for 30 s, and extension at 72C for 30 s.

Analysis of mycotoxin concentrations in feed

Deoxynivalenol was isolated from feed with the use of immunoaffinity columns (DON-Test™ Don Testing System VICAM, Watertown, USA). All extraction procedures were performed in accordance with the recommendations of the column manufacturer. Chromatographic analyses were conducted with the use of the Agilent 1100 series HPLC system equipped with 4.6 x 100 mm (3.5 fm) Eclipse Plus C[18] columns. The mobile phase was a water and acetonitrile mixture with a 90:10 solvent ratio. Flow rate was 0.6 ml/min. The mycotoxin was identified with the use of a diode detector at 220 nm wavelength.

Zearalenone was isolated from feed with the use of the Zearala-Test™ (VICAM, Watertown, USA). All extraction procedures were performed in accordance with the recommendations of the column manufacturer. Chromatographic analyses were conducted with the use of an Agilent 1100 series HPLC system in accordance with the procedure described by Zwierzchowski et al. (2004).

Table 1. Percentages of TLR2- and TLR9-positive cells, including mean values, standard deviation and P values, in the evaluated sections of the large intestine. * – significant at $p \leq 0.05$.

| Group		Ascending colon		Descending colon		Cecum	
Pig No.		E	C	E	C	E	C
TLR2	1	7.778	8.214	7.122	13.247	5.335	16.363
	2	7.8226	9.9639	22.429	12.882	28.791	11.093
	3	25.33	11.968	4.701	26.610	6.127	9.670
	4	10.535	6.218	6.123	4.198	7.547	5.244
	Mean	12.866	9.091	10.094	14.234	11.95	10.593
	SD	8.408	2.453	8.283	9.25	11.264	4.583
	p value	0.421		0.529		0.83	
Group		Ascending colon		Descending colon		Cecum	
Pig No.		E	C	E	C	E	C
TLR9	1	85.972	72.167	80.469	76.141	48.977	86.408
	2	84.508	70.964	64.26	78.457	74.152	88
	3	87.392	63.06	43.109	76.805	47.016	74.364
	4	76.392	79.874	60.371	73.22	64.261	69.912
	Mean	83.566*	71.516	62.052	76.156	58.602*	79.671
	SD	4.925	5.959	15.337	2.186	12.918	8.91
	p value	0.029		0.118		0.036	
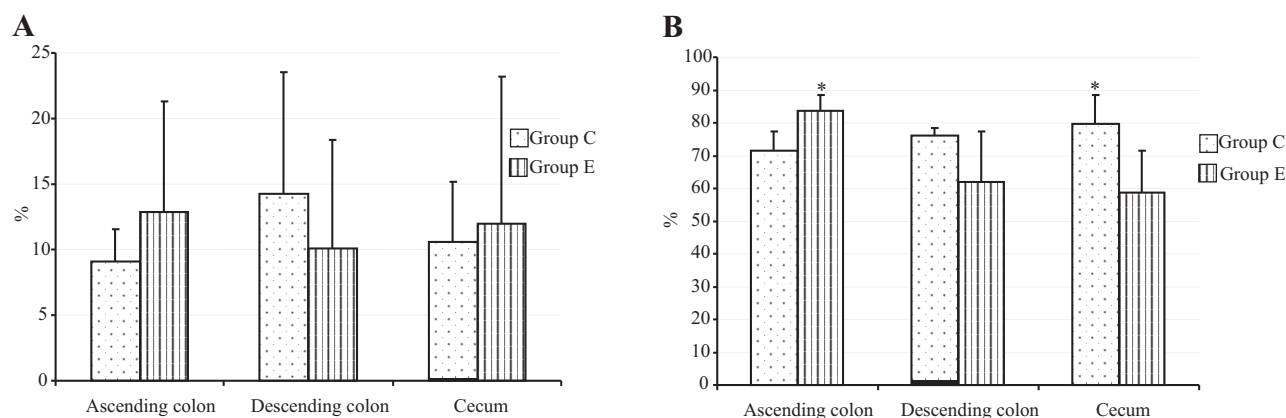
Fig. 1. A – Percentage of TLR2-positive enterocytes in the analyzed sections of the large intestine. B – Percentage of TLR9-positive enterocytes in the analyzed sections of the large intestine. * – significant at p≤0.05.

Fumonisins B_1 and B_2 were isolated with the use of the FumoniTest™ WB (VICAM, Watertown, USA). All extraction procedures were performed in accordance with the recommendations of the column manufacturer. Chromatographic analyses were conducted with the use of the Agilent 1100 series HPLC system, and fumonisin derivatives were obtained from o-phthaldialdehyde in the presence of mercaptoethanol. Separation was performed on a reversed phase C^{18} column. The mobile phase was a mixture of ethanol and 0.1 M sodium dihydrogen phosphate (80:20) with a pH of 3.35 (Wiśniewska-Dmytrow et al. 1999).

Ochratoxin A was isolated with the use of immunoaffinity columns (OchraTest™ WB Mycotoxin Testing System, VICAM, Watertown, USA) in accordance with the manufacturer's guidelines. Chromatographic analyses were conducted with the use of the Agilent 1100 series HPLC system. The analyzed extract, in the amount of 100 μl, was applied to the Poroshell RP C^{18} column. The elution mixture was composed of 0.25 N H_3PO_4, acetonitrile and 2-propanol (55:19:28), and the flow rate was set at 0.9 ml/min. Ochratoxin A was identified with a fluorometer at 330/460 nm wavelength (Czerwiecki et al. 2004).
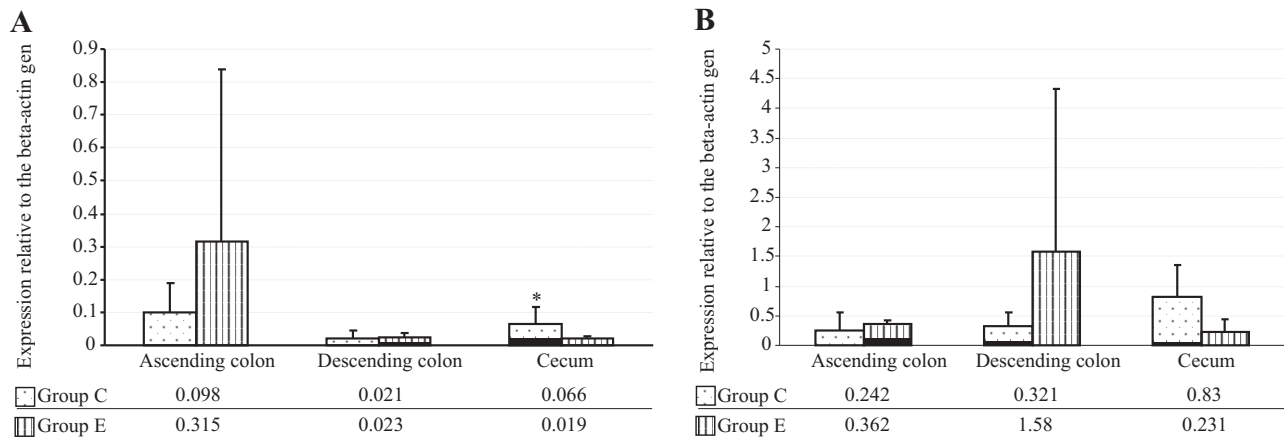
Fig. 2. A – Relative expression of TLR2 mRNA in the analyzed sections of the large intestine. B – Relative expression of TLR9 mRNA in the analyzed sections of the large intestine. * – significant at p≤0.05.

Statistical analysis

The percentage of enterocytes positive for TLR2 and TLR9 was expressed by mean values ($\bar{x}$) with standard deviation (SD). Data were processed using the Statistica program (StatSoft Inc., USA). The assumption that dependent variables in groups E and C have normal distribution was verified using the Kolmogorov-Smirnov test. The equality of variances between groups was checked using Levene's test. Differences between groups were determined using Student's t-test. The results were significant at $p \leq 0.05$ and highly significant at $p \leq 0.01$.

The relative expression of TLR2 and TLR9 mRNA in the examined segments of the large intestine was represented by mean values ($\bar{x}$) with standard deviation (SD). Data were processed using the Statistica program (StatSoft Inc., USA). The assumption that the analyzed parameters have normal distribution in groups E and C was ruled out using the Kolmogorov-Smirnov test. Statistically significant differences were identified by the Wilcoxon rank-sum test. The results were significant at $p \leq 0.05$ and highly significant at $p \leq 0.01$.

Results

Feed naturally contaminated with mycotoxin was supplied by a commercial feed mill. The presence of DON, zearalenone (ZEN), fumonisins B_1 (FB_1) and B_2 (FB_2), and ochratoxin (OTA) in feed was determined by HPLC-FLD/UV. Feed was contaminated with 1008 µg/kg DON. The remaining mycotoxins were below the limit of detection (LOD). The feed administered to group C was supplied by the same feed manufacturer. It was analyzed for the presence of DON, ZEN, FB_1, FB_2 and OTA. The results were below LOD values.

A significant ($p \leq 0.05$) increase in the percentage of TLR9-positive enterocytes was observed in the ascending colon in group E relative to group C. A significant ($p \leq 0.05$) decrease in the percentage of TLR9-positive cells was noted in the cecum in group E. Significant differences were not observed in the percentage of TLR9-positive enterocytes in the descending colon or in the percentage of TLR2-positive enterocytes in all analyzed segments of the large intestine. The percentages of TLR2- and TLR9-positive cells, including mean values, SD values and *p* values in groups E and C in the investigated sections of the large intestine, are presented in Table 1 and Fig. 1.

A statistical analysis of the relative expression of TLR2 and TLR9 mRNA revealed a significant ($p \leq 0.05$) decrease in TLR2 levels in the cecum in group E. Significant differences in gene expression were not observed in the remaining segments of the large intestine. Mean values with standard deviation for TLR2 and TLR9 are presented in Fig. 2.

Discussion

The aim of this study was to determine the influence of feed contaminated with DON at levels similar to the reference doses recommended by the European Commission on the expression of TLR2 and TLR9 mRNA and the percentage of TLR2- and TLR9-positive enterocytes in the porcine large intestine.

Real-time PCR revealed a significant decrease in the relative expression of TLR2 in the cecum. Immunohistochemical evaluation of the number of TLR2- and TLR9-positive enterocytes demonstrated that the percentage of TLR9-positive cells increased significantly in the ascending colon and decreased in the cecum.

The results of this study indicate that DON can modify the local immune response by changing the expression of TLR2 and TLR9 in the large intestine.

There is growing evidence to indicate that porcine tissues harbor various TLRs whose expression is influenced by a host of biological and chemical factors. There is a general scarcity of published data relating to the effect of mycotoxins on the expression and reactivity of TLRs *in vivo*.

Uddin et al. (2013) observed that mRNA expression patterns in all members of the TLR family in the porcine gut increases with age because gut-associated lymphoid tissue (GALT) is continuously exposed to commensal and pathogenic microflora, which creates a balance between tolerance and immunity. These authors also noted that the levels of TLR2, TLR3 and TLR9 proteins in the porcine gut do not always correspond to mRNA expression levels, which is consistent with our findings. In contrast, Hausmann et al. (2002) reported that macrophages isolated from humans with inflamed intestinal mucosa were characterized by higher levels of TLR2 and TLR4 proteins and mRNA expression. A study of the IPEC-1 cell line exposed to ZEN demonstrated an increase in the mRNA expression of genes encoding TLR1-TLR10 and inflammatory cytokines, but only in the presence of the *Escherichia coli* K88 ligand (Taranu et al. 2015). In an *in vitro* study of the RAW 264.7 mouse macrophage cell line, Pestka and Zou (2006) observed that cell incubation with LPS, the TLR4 ligand, increased macrophage sensitivity to DON, which elevated the expression of IL-1β, IL-6 and TNFα mRNA in the evaluated cells. Somewhat different results were reported by Sugiyama et al. (2016) in an *in vitro* study of macrophages stimulated with LPS where DON decreased the expression of myeloid differentiation factor 88 (MyD88), the adaptor protein for most TLRs which induces nuclear factor κB (NF-κB), the protein responsible for macrophage activation and inflammatory states.

Numerous studies investigating TLR expression in the large intestine have been performed on patients diagnosed with inflammatory bowel disease (IBD). It is believed that IBD is caused by abnormal gut microflora, increased intestinal permeability and changes in TLR expression (Kassinen et al. 2007, Camilleri and Gorman 2007, Brint et al. 2011). Belmonte et al. (2012) demonstrated that IBD patients suffering from constipation alternating with diarrhea had higher TLR2 and TLR4 expression in colonic enterocytes. Mice with turned off TLR2 gene were more sensitive to colitis induced by dextran sulfate sodium (DSS) (Cario et al. 2010). Ligand-specific stimulation of TLR2 activates PKC-α/δ, which increases TEER and leads to the redistribution of ZO-1, the key tight junction (TJ) component (Cario 2010). Toll-like receptor 9 also plays a key role in IBD. Sánchez-Muñoz et al. (2011) reported higher expression of TLR9 mRNA in 51 patients diagnosed with ulcerative colitis relative to healthy subjects. A study of mice with induced colitis also revealed that the TLR9 ligand intensifies inflammatory states (Obermeier et al. 2002). In contrast, CpG oligonucleotides alleviated symptoms of colitis in mice with turned off IL-10 gene (Rachmilewitz et al 2002). Rose et al. (2012) also reported a higher risk of colitis and significantly slower intestinal healing in mice with turned off TLR9 gene.

The current state of knowledge does not explain which types of changes related to TLR expression in the intestines are implicated in IBD. It can be assumed that the entire spectrum of disorders associated with IBD is characterized by abnormal TLR expression. The described *in vivo* experiment was performed on a porcine model where enterocytes were continuously exposed to intestinal microflora and DON. The number of TLR9-positive cells increased in the ascending colon and decreased in the cecum. The expression of TLR2 mRNA decreased in the cecum. Our findings suggest that DON is able to modify the expression of TLR2 and TLR9 mRNA *in vivo*. The analyzed mycotoxin decomposes intestinal microflora (Piotrowska et al. 2014), decreases TEER and increases intestinal permeability (Pinton and Oswald 2014), which could indicate that it induces and/or exacerbates immune disorders characteristic of IBD. Our results are relevant for future research which should focus on mycotoxin influence on the family of TLR proteins and the profile of proinflammatory and anti-inflammatory cytokines in the gastrointestinal tract.

References

Abysique A, Tardivel C, Troadec JD, Felix B (**2015**) The food contaminant mycotoxin deoxynivalenol inhibits the swallowing reflex in anaesthetized rats. PLoS One 10: e0133355.

Akira S, Takeda K (**2004**) Toll-like receptor signaling. Nat Rev Immunol 4: 499-511.

Belmonte L, Beutheu Youmba S, Bertiaux-Vandalle N, Antonietti M, Lecleire S, Zalar A, Gourcerol G, Leroi AM, Déhelotte P, Coéfier M, Ducrotté P (**2012**) Role of toll like receptors in irritable bowel syndrome: differential mucosal immune activation according to the disease subtype. PLoS One 7: e42777.

Binder EM (**2007**) Managing the risk of mycotoxins in modern feed production. Anim Feed Sci Technol 133: 149-166.

Bouhet S, Oswald IP (**2005**) The effects of mycotoxins, fungal food contaminants, on the intestinal epithelial cell-derived innate immune response. Vet Immunol Immunopathol 108: 199-209.

Brint EK, MacSharry J, Fanning A, Shanahan F, Quigley EM (**2011**) Differential expression of toll-like receptors in patients with irritable bowel syndrome. Am J Gastroenterol 106: 329-336.

Bryden WL (**2007**) Mycotoxins in the food chain: human health implications. Asia Pac J Clin Nutr 16 (Suppl 1): 95-101.

Burkey TE, Skjolaas KA, Dritz SS, Minton JE (**2009**) Expression of porcine Toll-like receptor 2, 4 and 9 gene transcripts in the presence of lipopolysaccharide and Salmonella enterica serovars Typhimurium and Choleraesuis. Vet Immunol Immunopathol 130: 96-101.

Camilleri M, Gorman H (**2007**) Intestinal permeability and irritable bowel syndrome. Neurogastroenterol Motil 19: 545-552.

Cario E, Gerken G, Podolsky DK (**2007**) Toll-like receptor 2 controls mucosal inflammation by regulating epithelial barrier function. Gastroenterology 132: 1359-1374.

Cario E (**2010**) Toll-like receptors in inflammatory bowel diseases: a decade later. Inflamm Bowel Dis 16: 1583-1597.

Chung YJ, Zhou HR, Pestka JJ (**2003**) Transcriptional and posttranscriptional roles for p38 mitogen-activated protein kinase in upregulation of TNF-alpha expression by deoxynivalenol (vomitoxin). Toxicol Appl Pharmacol 193: 188-201.

Czerwiecki L, Czyżyk K, Kwiecińska A, Wilczyńska G (**2004**) Immunoaffinity columns and determination of ochratoxin A in cereals by HPLC Part I.: evaluation of extraction using methanol/water. Roczn Panstw Zakl Hig 55: 133-138.

EC (**2006**) Commision recomendation on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. Official Journal of the European Union L229/7-L229/9 (http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CE LEX%3A32006H0576).

Feinberg B, McLaughlin CS (**1989**) Biochemical mechanism of action of tricothecene mycotoxins. In: Beasley VR (ed) Trichothecene Mycotoxicosis: Pathological Effects. CRC Press Inc., Boca Raton, FL, pp 27-35.

Fink-Gremmels J (**1999**) Mycotoxins: their implications for human and animal health. Vet Q 21: 115-120.

Hausmann M, Kiessling S, Mestermann S, Webb G, Spöttl T, Andus T, Schölmerich J, Herfarth H, Ray K, Falk W, Rogler G (**2002**) Toll-like receptors 2 and 4 are up-regulated during intestinal inflammation. Gastroenterology 122: 1987-2000.

He K, Pestka JJ (**2010**) Deoxynivalenol-induced modulation of microRNA expression in RAW 264.7 macrophages-A potential novel mechanism for translational inhibition. Toxicologist 114 (Toxicol Sci Suppl): 310.

Iordanov MS, Pribnow D, Magun JL, Dinh TH, Pearson JA, Chen SL, Magun BE (**1997**) Ribotoxic stress response: activation of the stress-activated protein kinase JNK1 by inhibitors of the peptidyl transferase reaction and by sequence-specific RNA damage to the alpha-sarcin/ricin loop in the 28S rRNA. Mol Cell Biol 17: 3373-3381.

Kassinen A, Krogius-Kurikka L, Mαkivuokko H, Rinttilα T, Paulin L, Corander J, Malinen E, Apajalahti J, Palva A (**2007**) The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. Gastroenterology 133: 24-33.

Kolf-Clauw M, Castellote J, Joly B, Bourges-Abella N, Raymond-Letron I, Pinton P, Oswald IP (**2009**) Development of a pig jejunal explant culture for studying the gastrointestinal toxicity of the mycotoxin deoxynivalenol: histopathological analysis. Toxicol In Vitro 23: 1580-1584.

Latz E, Schoenemeyer A, Visintin A, Fitzgerald KA, Monks BG, Knetter CF, Lien E, Nilsen NJ, Espevik T, Golenbock DT (**2004**) TLR9 signals after translocating from the ER to CpG DNA in the lysosome. Nat Immunol 5: 190-198.

Li M, Pestka JJ (**2008**) Comparative induction of 28S ribosomal RNA cleavage by ricin and the trichothecenes deoxynivalenol and T-2 toxin in the macrophage. Toxicol Sci 105: 67-78.

Meurens F, Summerfield A, Nauwynck H, Saif L, Gerdts V (**2012**) The pig: a model for human infectious diseases. Trends Microbiol 20: 50-57.

Middlebrook JL, Leatherman DL (**1989**) Binding of T-2 toxin to eukaryotic cell ribosomes. Biochem Pharmacol 38: 3103-3110.

Obermeier F, Dunger N, Deml L, Herfarth H, Scholmerich J, Falk W (**2002**) CpG motifs of bacterial DNA exacerbate colitis of dextran sulfate sodium-treated mice. Eur J Immunol 32: 2084-2092.

Patterson JK, Lei XG, Miller DD (**2008**) The pig as an experimental model for elucidating the mechanisms governing dietary influence on mineral absorption. Exp Biol Med (Maywood) 233: 651-664.

Pestka JJ, Smolinski AT (**2005**) Deoxynivalenol: toxicology and potential effects on humans. J Toxicol Environ Health B Crit Rev 8: 39-69.

Pestka JJ, Zhou HR (**2006**) Toll-like receptor priming sensitizes macrophages to proinflammatory cytokine gene induction by deoxynivalenol and other toxicants. Toxicol Sci 92: 445-455.

Pestka JJ (**2010**) Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Arch Toxicol 84: 663-679.

Pinton P, Oswald IP (**2014**) Effect of deoxynivalenol and other Type B trichothecenes on the intestine: a review. Toxins (Basel) 6: 1615-1643.

Piotrowska M, Sliżewska K, Nowak A, Zielonka L, Zakowska Z, Gajęcka M, Gajęcki M (**2014**) The effect of experimental fusarium mycotoxicosis on microbiota diversity in porcine ascending colon contents. Toxins (Basel) 6: 2064-2081.

Rachmilewitz D, Karmeli F, Takabayashi K, Hayashi T, Leider-Trejo L, Lee J, Leoni LM, Raz E (**2002**) Immunostimulatory DNA ameliorates experimental and spontaneous murine colitis. Gastroenterology 122: 1428-1441.

Rodrigues I, Naehrer K (**2012**) A three-year survey on the worldwide occurrence of mycotoxins in feedstuffs and feed. Toxins (Basel) 4: 663-675 .

Rose WA 2nd, Sakamoto K, Leifer CA (**2012**) TLR9 is important for protection against intestinal damage and for intestinal repair. Sci Rep 2: 574.

Sanchez-Muñoz F, Fonseca-Camarillo G, Villeda-Ramtrez MA, Miranda-Pérez E, Mendivil EJ, Barreto-Zúñiga R, Uribe M, Bojalil R, Domínguez-López A, Yamamoto-Furusho JK (**2011**) Transcript levels of Toll-Like Receptors 5, 8 and 9 correlate with inflammatory activity in Ulcerative Colitis. BMC Gastroenterol 11: 138.

Sergent T, Parys M, Garsou S, Pussemier L, Schneider YJ, Larondelle Y (**2006**) Deoxynivalenol transport across human intestinal Caco-2 cells and its effects on cellular metabolism at realistic intestinal concentrations. Toxicol Lett 164: 167-176.

Shifrin VI, Anderson P (**1999**) Trichothecene mycotoxins trigger a ribotoxic stress response that activates c-Jun N-terminal kinase and p38 mitogen-activated protein kinase and induces apoptosis. J Biol Chem 274: 13985-13992.

Shimosato T, Tohno M, Kitazawa H, Katoh S, Watanabe K, Kawai Y, Aso H, Yamaguchi T, Saito T (**2005**) Toll-like receptor 9 is expressed on follicle-associated epithelia containing M cells in swine Peyer's patches. Immunol Lett 98: 83-89.

Sugiyama K, Muroi M, Kinoshita M, Hamada O, Minai Y, Sugita-Konishi Y, Kamata Y, Tanamoto K (**2016**) NF-κB activation via MyD88-dependent Toll-like receptor signaling is inhibited by trichothecene mycotoxin deoxynivalenol. J Toxicol Sci 41: 273-279.

Taranu I, Marin DE, Pistol GC, Motiu M, Pelinescu D (**2015**) Induction of pro-inflammatory gene expression by Escherichia coli and mycotoxin zearalenone contamination and protection by a Lactobacillus mixture in porcine IPEC-1 cells. Toxicon 97: 53-63.

Thoma-Uszynski S, Kiertscher SM, Ochoa MT, Bouis DA, Norgard MV, Miyake K, Godowski PJ, Roth MD, Modlin RL (**2000**) Activation of Toll-like receptor 2 on human dendritic cells triggers induction of IL-12, but not IL-10. J Immunol 165: 3804-3810.

Tohno M, Shimosato T, Kitazawa H, Katoh S, Iliev ID, Kimura T, Kawai Y, Watanabe K, Aso H, Yamaguchi T, Saito T (**2005**) Toll-like receptor 2 is expressed on the intestinal M cells in swine. Biochem Biophys Res Commun 330: 547-554.

Uddin MJ, Kaewmala K, Tesfaye D, Tholen E, Looft C, Hoelker M, Schellander K, Cinar MU (**2013**) Expression patterns of porcine Toll-like receptors family set of genes (TLR1-10) in gut-associated lymphoid tissues alter with age. Res Vet Sci 95: 92-102.

Uenishi H, Shinkai H (**2009**) Porcine Toll-like receptors: the front line of pathogen monitoring and possible implications for disease resistance. Dev Comp Immunol 33: 353-361.

Wetzler LM (**2003**) The role of Toll-like receptor 2 in microbial disease and immunity. Vaccine 21 (Suppl 2): S55-S60.

Wiśniewska-Dmytrow H, Kozak A, Żmudzki J (**1999**) Determination of fumonisins B1 and B2 in corn and fodder by liquid chromatography. Med Weter 55: 114-117.

Zhou HR, Lau AS, Pestka JJ (**2003**) Role of double-stranded RNA-activated protein kinase R (PKR) in deoxynivalenol-induced ribotoxic stress response. Toxicol Sci 74: 335-344.

Zwierzchowski W, Gajęcki M, Obremski K, Zielonka Ł, Baranowski M (**2004**) The occurrence of zearalenone and its derivatives in standard and therapeutic feeds for companion animals. Pol J Vet Sci 7: 289-293.