Effect of the mycotoxin deoxynivalenol on the immune responses of rainbow trout (*Oncorhynchus mykiss*)

I. Matejova[1,2], M. Vicenova[2], L. Vojtek[3], H. Kudlackova[2], K. Nedbalcova[2], M. Faldyna[2], E. Sisperova[1], H. Modra[1], Z. Svobodova[1]

[1]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
[2]Veterinary Research Institute, Brno, Czech Republic
[3]Masaryk University, Brno, Czech Republic

ABSTRACT: The trichothecene mycotoxin deoxynivalenol (DON) is commonly found as a natural contaminant in cereals such as wheat, barley, and corn, and exhibits various toxicological effects when present in animal feeds. The effects of DON at a nominal 2 mg/kg feed on immune responses of rainbow trout were investigated, including relative gene expression of important cytokines (TNF-α, IL-8, IL-1β, IL-10), lysozyme concentration in skin mucus, and level of antigen-specific IgM in blood plasma after vaccination with the commercial vaccine AquaVac ERM containing *Yersinia ruckeri* type 1 (Hagerman strain). Twenty one-year-old rainbow trout *Oncorhynchus mykiss* were randomly divided into two groups. The control received a commercial feed with a naturally occurring low level of DON (225 µg/kg feed), while an experimental group was fed the same formulation with DON added to 1964 µg/kg feed. The trial continued for 23 days. Consumption of feed with added DON showed a significant effect on the immune system, as indicated by a higher level of pro-inflammatory cytokine TNF-α ($P < 0.05$) and of IL-8 (non-significant) in head kidney. Expression of the pro-inflammatory gene IL-1β and the expression of a gene encoding anti-inflammatory cytokine (IL-10) were not influenced by DON treatment. Effects on the concentration of skin mucus lysozyme and specific IgM antibody levels were not observed during this experiment. These results suggest that prolonged ingestion of low doses of DON may influence the immune responses of rainbow trout.

Keywords: fish; feedstuffs; mycotoxin; immune system

Abbreviations

DON = deoxynivalenol; **IgM** = immunoglobulin M; **IL** = interleukin; **L : D** = light : dark; **TNF-α** = tumour necrosis factor alpha

Mycotoxins are secondary metabolites produced by some fungal genera, primarily *Aspergillus*, *Penicillium*, *Fusarium* and *Claviceps*. Deoxynivalenol (DON), also known as vomitoxin, is mainly produced by *Fusarium graminearum* and *Fusarium culmorum*. This toxin is resistant to milling and processing and is not degraded by high temperatures (Sugita-Konishi et al. 2006).

Although not the most toxic, DON is considered to be the most economically important mycotoxin, since it is the predominant trichothecene contaminant in cereal crops such as wheat, barley, and corn used for human consumption and in animal feed (Wegulo 2012). DON contamination of animal feed components and finished feeds is common (Streit et al. 2012). The potential for mycotoxin contamination of aquaculture feed depends to some extent on plant material quality and the amount of plant-sourced ingredients in the feed (Manning and Abbas 2012). Over the past decade, reduced availability of fish meal has led to increasing use of cereal grains, especially wheat, as a source of protein in commercial aqua-

culture feeds. Feed formulations for rainbow trout range from 15–27% wheat. For herbivorous species such as cyprinids, the amount of wheat is higher, from 20–70% (Pietsch et al. 2013).

In animals, DON causes a variety of toxic and immunotoxic effects depending on dose and duration of exposure, age, species, and health and nutritional status (Pestka et al. 2004). The toxicity of DON is well recognised in mammals, and research on its toxicity to fish and other aquatic organisms is increasing. A recent investigation of zebrafish *Danio rerio* fed DON at concentrations of 0.1–3 mg/kg feed showed higher levels of genetic liver biomarkers and an influence on the reproductive system (Sanden et al. 2012). In a previous study we found changes in haematological and biochemical parameters, including pathological alterations in caudal kidney, of rainbow trout fed DON at 2 mg/kg feed (Matejova et al. 2014). Woodward et al. (1983) found that diets containing 1.0–12.9 mg/kg DON depressed feed intake and weight gain and decreased feed conversion efficiency of juvenile rainbow trout. Similarly, a study by Hooft et al. (2011) on salmonids showed intake of increasing levels of DON at concentrations of 0.3–2.6 mg/kg feed to be associated with a significant decrease in feed intake, weight gain, and growth rate along with patho-morphological changes of the liver, including subcapsular haemorrhage, subcapsular oedema, and fatty infiltration of the hepatocytes. Their results showed rainbow trout to be more sensitive to DON compared to other fish species. This difference in sensitivity may be due to differing ability of the intestinal microbes to transform DON into less toxic deepoxy DON (dE-DON) (Guan et al. 2009). Cyprinids appear to tolerate DON at concentrations of 0.4–1 mg/kg feed, as was also observed in zebrafish (Sanden et al. 2012; Pietsch et al. 2014).

The characteristic responses seen in other animals after acute exposure to high doses of DON are vomiting and diarrhoea, whereas prolonged low dose feeding of DON is associated with retarded weight gain, anorexia, reduced feed conversion, gastrointestinal haemorrhaging, inflammation, and immune system alterations (Bracarense et al. 2012). Immune cells are a primary target for this mycotoxin. The impact of DON on the immune system ranges from immunosuppression to immunostimulation. Low exposure to DON leads to stimulation of immunity, up-regulating the production of cytokines and expression of inflammatory genes, whereas high concentrations cause leukocyte apoptosis and suppression of immune responses. The main effect at the cellular level is inhibition of protein synthesis by binding to the peptidyl transferase region of ribosomes (Pestka et al. 2004).

The effects of DON on the immune system of fish have rarely been investigated. Mycotoxin-induced alterations in immune function may predispose fish to infectious diseases and pose a threat to fish in aquaculture. The present study was aimed at evaluating the effects of an experimentally DON-contaminated diet on the immune responses of rainbow trout (*Oncorhynchus mykiss*).

MATERIAL AND METHODS

Experimental protocol. The experimental protocol was approved by the Branch Commission for Animal Welfare of the Ministry of Education, Youth, and Sports of the Czech Republic (Ref. No. MSMT 18977/2013-1). The study was carried out with 20 clinically healthy one-year-old rainbow trout obtained from a commercial fish farm. Fish were randomly assigned to 200 l tanks (five per tank) with flow-through aerated dechlorinated water and maintained under a 12 : 12 L : D photoperiod. The aquaria were cleaned daily by siphoning off the faeces and debris.

Water quality parameters throughout the acclimation and exposure period were as follows: dissolved oxygen 80.5–95.2%, pH 7.9–8.2, temperature 14.0–15.1 °C. The physico-chemical water parameters were total ammonia 0.1–0.5 mg/l, NO_3^- 20 to 30 mg/l, NO_2^- 0.1–0.4 mg/l, and Cl^- 20–25 mg/l.

Rainbow trout were acclimatised to laboratory conditions for 14 days, during which time they were fed a commercial rainbow trout diet (BioMar, Denmark) twice daily at 1% of body mass. After the adaptation period, trout were randomly divided into control (*n* = 10; two aquariums per five fish) and experimental (*n* = 10; two aquariums per five fish) groups and fed their respective diet twice daily.

To determine whether exposure to DON can influence animal response to vaccine, nine days after the beginning of the experiment fish in both groups were treated by a single immersion in an AquaVac ERM (Schering-Plough Animal Health, UK) containing *Yersinia ruckeri* type 1 (Hagerman strain) diluted 1 : 10 for 30 s according to the manufacturer's instruction. The trial was terminated at

23 days. No morbidity and mortality was recorded during the study. The same animals were used as in an already published study (Matejova et al. 2014).

To evaluate lysozyme concentration, skin mucus samples were taken at the end of the experiment. Blood samples for detection of IgM-specific antibodies were taken from each fish by caudal venipuncture and stabilised with sodium heparin (50 IU/ml of blood). Trout were subsequently stunned with a blow to the head and killed by spinal transection. Samples of head kidney and spleen were taken for real-time PCR.

Experimental diet. The commercial diet (BioMar, Denmark) contained rapeseed oil, blood meal, fish meal, soya cake, sunflower cake, rapeseed meal, horse beans, wheat, soya concentrate, fish oil, pea protein, vitamins, and minerals based on the manufacturer's information. The experimental diet contained DON (Sigma-Aldrich, USA) at 2 mg/kg, as previously described (Matejova et al. 2014). The leaching of DON from experimental diets probably did not influence its intake, as fish in all groups consumed the pellets within 20 min.

Analysis of mycotoxin. In naturally contaminated feeds there may be additional toxins such as acetylated derivates of DON (3-ADON, 15-ADON) or other mycotoxins (Pinton et al. 2012). In the present study, the levels of deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, diacetoxyscirpenol, fumonisin B_1 and B_2, HT-2 toxin, T-2 toxin, nivalenol, ochratoxin A, and zearalenone were determined in the control and experimental diets using liquid chromatography-tandem mass spectrometry (LC-MS/MS) by the Metrology and Testing Laboratory (Institute of Chemical Technology, Czech Republic). Deoxynivalenol was found to be naturally present in the commercial diet at 225 μg/kg feed. Natural contamination of the control diet was comparable to average DON content in commercially available feeds (Streit et al. 2012). In the experimental diet, 1964 μg/kg feed of DON and 1 μg/kg feed of zearalenone were

detected. All other mycotoxins were below the limits of detection.

Quantitative real-time RT-PCR (qRT-PCR) detection of cytokines. Samples of head kidney and spleen tissue were removed from six randomly selected fish and immediately stabilised in RNAlater (Qiagen, Germany), kept at 4 °C for 24 h, and subsequently frozen at –80 °C. Samples were removed from the stabilisation reagent and lysed in 1 ml TRI reagent RT (Molecular Research Center, USA) and homogenised on a MagNA Lyser (Roche, Switzerland) with silica beads (BioSpec Products, USA). After 4-Bromoanisole phase separation in the TRI Reagent, the total RNA was isolated and purified using the RNeasy Kit (Qiagen, Germany) according to the manufacturer's instructions. Reverse transcription of RNA was carried out using M-MLV reverse transcriptase (200 IU) (Invitrogen, UK) and oligo-dT primers at 37 °C for 1.5 h. The cDNA was stored at –20 °C until PCR analysis. qRT-PCR was performed with the LightCycler 480 (Roche) using Quan-tiTect SYBR Green PCR Kit (Qiagen, Germany). Primers for 4 cytokines (IL-1β, IL-8, TNF-α, IL-10) and two candidate reference genes (60S, β-actin) were used (Table 1), adapted from Perez-Sanchez et al. (2011). Using the RefFinder tool (http://www.leonxie.com/reference-gene.php), the 60S gene was selected for normalisation of expression data. The relative expression of the gene of interest (GOI) was calculated according to the formula [1/2^Ct(GOI)]/[1/2Ct(reference gene)] (Zelnickova et al. 2008).

Specific IgM antibody levels. Blood samples were obtained from the caudal vein of each fish and stabilised with sodium heparin (50 IU/ml of blood). A sample of heparinised blood (2 ml) was centrifuged at 855 × g for 10 min in a cooled centrifuge (4 °C) and used to detect IgM-specific antibodies in blood plasma with a homemade ELISA kit.

To prepare antigens for coating microtitre plates, *Y. ruckeri* (CCM 6093, frozen at –80 °C) was cul-

Table 1. Nucleotide sequences of primers for real-time PCR

Gene	Forward primer 5'-3'	Reverse primer 3'-5'
TNF-α	GGGGACAAACTGTGGACTGA	GAAGTTCTTGCCCTGCTCTG
IL-8	AGAATGTCAGCCAGCCTTGT	TCTCAGACTCATCCCCTCAGT
IL-1β	ACATTGCCAACCTCATCATCG	TTGAGCAGGTCCTTGTCCTTG
IL-10	CGACTTTAAATCTCCCATCGAC	GCATTGGACGATCTCTTTCTTC
60S	AGCCACCAGTATGCTAACCAGT	TGTGATTGCACATTGACAAAAA
β-actin	ACAGACTGTACCCATCCCAAAC	AAAAAGCGCCAAAATAACAGAA

tivated in Petri dishes containing Tryptic soy agar (BD Difco, USA) at 25 °C for three days. After three days, the bacterial cultures were passaged and cultured for a further three days. After the first passage, the colonies were washed in sterile PBS and bacterial cells were subsequently sonicated (BANDELIN, Sonopuls HD 3100, Germany) and centrifuged at 9500 × g for 10 min at 10 °C. The total protein concentration was determined using the bicinchoninic acid (BCA) protein assay (Pierce BCA Protein Assay Kit, Thermo Scientific, USA).

Microtitre plates (Nunc MaxiSorp, Denmark) were coated with the antigen dissolved in carbonate-bicarbonate buffer (3 μg/ml per well), pH 9.6. The plates were incubated for 15 h at 4 °C and subsequently rinsed five times with a diluting solution containing PBS, pH 7.2, and 0.05% Tween 20. Subsequently, a solution of 0.5% casein + 10% sucrose was added and held for 30 min. Tested sera were prediluted 1 : 10 in diluting solution (PBS, pH 7.2; 0.05% Tween 20; 0.5% casein hydrolysate). After 60 min of incubation at room temperature (20 °C), the wells were rinsed five times and 100 μl of monoclonal antibody anti-rainbow trout IgM (Aquatic Diagnostic Ltd., Scotland) was added. The plates were incubated for 1 h at room temperature, rinsed five times and anti-mouse IgG horseradish peroxidase-labelled conjugate (Bethyl Laboratories, USA) diluted 1 : 3000 was added to each well. After a further hour of incubation at room temperature (20 °C), the plates were rinsed five times and 100 μl of substrate + chromogen TMB mixture (Test-line, Czech Republic) was added to each well. After a 15 min incubation, the reaction was stopped by adding 100 μl of 2M sulphuric acid, and absorbances were read at 450 nm using a multichannel spectrometer, Synergy H1 (BioTek, USA).

The assay was further characterised by validation parameters: The coefficient of the variation for homogeneity of the microplate rating was 4.52%; the intra-assay and inter-assay coefficients of variations were 5.63 and 11.79%, respectively.

Lysozyme concentration. The lysozyme concentration in skin mucus was determined *in vitro* by radial diffusion in agarose (Poisot et al. 2009). Skin mucus samples were collected by gently scraping the dorso-lateral surface from the fish before blood collection using the blunt edge of a scalpel. The mucus samples (15 μl) or standard solutions were placed in a well cut into the agarose containing inactivated Gram-positive bacteria *Micrococcus luteus* (CCM 169). Plates with gels were incubated

in a wet box at room temperature (20 °C) for 24 h. Diameters of lytic zones were recorded, and the concentrations of lysozyme (mg/ml) were calculated using a calibration curve equation.

Statistical analysis. Data were assessed using the unpaired nonparametric Mann-Whitney U test. The statistical software GraphPad Prism 5 (GraphPad Software, USA) was used for the statistical analysis. Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

Cytokine expression

Using real-time PCR, relative gene expression of four important cytokines including pro-inflammatory cytokines (TNF-α, IL-1β, IL-8) and an anti-inflammatory cytokine (IL-10) was detected in rainbow trout head kidney and spleen (Figure 1). In each sample, the housekeeping gene 60S was used. Genes for TNF-α and IL-8 were up-regulated in head kidney; the difference in TNF-α of control and experimental groups was statistically signifi-
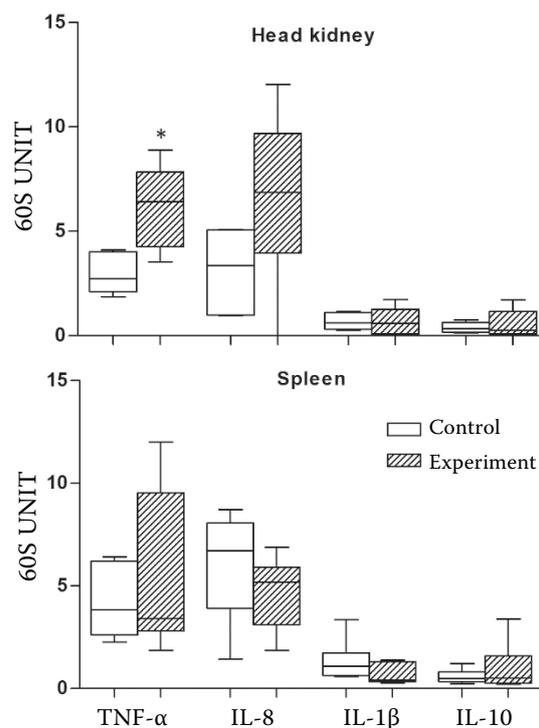

Figure 1. Relative mRNA expression in rainbow trout head kidney and spleen versus reference gene; 60S, *n* = 6. Results are given as the second and third quartiles (boxes), minimum and maximum (whisker limits), and median; *$P < 0.05$
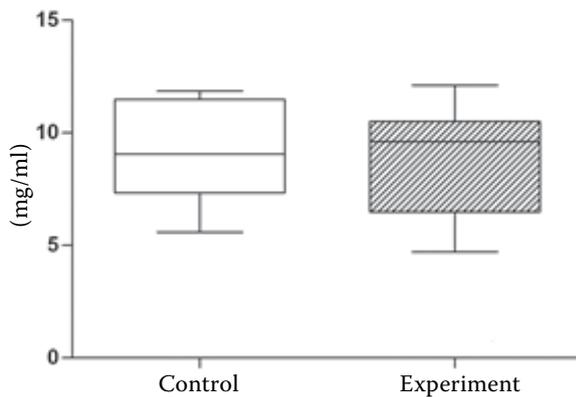
Figure 2. Concentration (mg/ml) of lysozyme in skin mucus of control and experimental rainbow trout, *n* = 10. Results are given as the second and third quartiles (boxes), minimum and maximum (whisker limits), and median
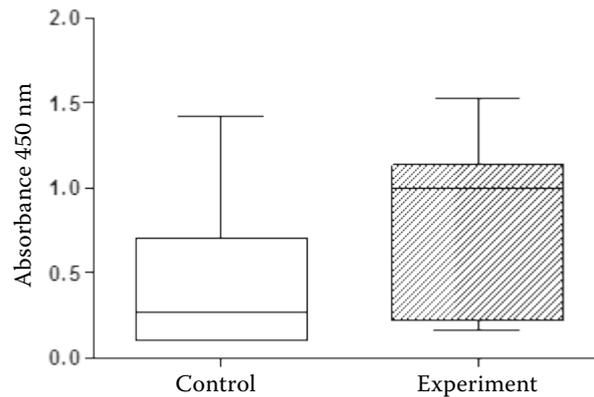

Figure 3. Levels of specific IgM reaction with *Yersinia ruckeri* type 1 (Hagerman strain) in rainbow trout plasma. Results are given as the second and third quartiles (boxes), minimum and maximum (whisker limits), and median

cant. The other cytokines in head kidney were not significantly changed after the 23 day exposure. No significant difference in mRNA expression in spleen was found between control and experimental groups.

Lysozyme concentration

No significant differences between groups were observed in lysozyme activity in skin mucus (Figure 2).

Specific IgM antibody levels

Specific IgM levels were analysed 14 days post-vaccination in experimental and control groups using an enzyme-linked immunosorbent assay (ELISA). The antibody levels in experimental fish did not significantly differ from the control group (Figure 3).

DISCUSSION

The toxicity of DON to the immune system is well recognised in mammals (Fink-Gremmels 2008; Wache et al. 2009; Pestka 2010). Low levels of DON exposure up-regulate the expression of early response and pro-inflammatory genes, whereas high doses result in immune suppression through leukocyte apoptosis (Pestka et al. 2004).

Primary immune targets of this mycotoxin are monocytes and macrophages of the innate immune system. A single study has investigated the innate immune responses of carp *Cyprinus carpio* L. fed diets with DON content ranging from 0.4–1 mg/kg for 28 days. The results showed that pro-inflammatory (NO and ROS production) immune responses were stimulated by DON. The anti-inflammatory (arginase activity) immune responses were not significantly changed (Pietsch et al. 2014).

In macrophages, DON up-regulates mRNA for TNF-α and IL-6, and in monocytes activates IL-8 (Pestka 2010). In the current study, both pro-inflammatory and anti-inflammatory immune reactions were identified, revealing that pro-inflammatory immune responses were influenced by DON. The effects included up-regulation of TNF-α and IL-8 mRNA levels in head kidney of experimental fish; in the case of TNF-α, the up-regulation was statistically significant. The head kidney in teleosts is a major site of lymphoid tissue. It produces mainly macrophages and lymphoid cells, and is important for the capture of antigens and the production of antibodies (Secombes 2008). TNF-α is an important early response pro-inflammatory cytokine, and the level of its expression can indicate the presence of an inflammatory response (Press and Evensen 1999). This increase of TNF-α may suggest that the exposure to DON induces an acute phase reaction, which may be associated with cell death induced by DON. Vandenbroucke et al. (2011) described a dose-dependent decrease of cell viability when the immortalised cell line IPEC-J2 was exposed to different concentrations of DON. Other mechanisms leading to DON-induced inflammatory reactions include a direct stimulation of monocytes/

macrophages via activation of mitogen-activated protein kinases, which then drive up-regulation of cytokines or chemokine mRNA expression (Chung et al. 2003).

Teleost B cells produce three immunoglobulin isotypes, IgM, IgD, and IgT. IgM is the predominant molecule and principal player in systemic immunity in plasma, bile, and skin mucus (Esteban 2012). For the detection of the salmonid antibody response to the antigen *Y. ruckeri*, an enzyme-linked immunosorbent assay based on monoclonal antibody (Mab) was used. Ingestion of the DON-contaminated diet did not significantly change the level of the specific antibody IgM.

The final parameter measured in our study was the concentration of lysozyme in skin mucus. In vertebrates, the sites of the initial encounters of the immune system with antigens are physiological barriers, and in fish the important barrier to the entry of pathogens is the skin. Fish skin secretes mucus that contains several lytic enzymes and leukocytes responsible for local immune responses. Lysozyme is an important defence molecule of the innate immune system that catalyses the hydrolysis of peptidoglycan found in bacterial cell walls. It represents the key molecule in fish skin mucus; therefore, lysozyme concentration in skin mucus is an important indicator of the overall antibacterial status of the fish (Ondrackova et al. 2012). Variation in lysozyme activity in skin mucus could be influenced by factors such as season, species, sex, and diet (Balfry and Iwama 2014). In our study, the primary interest was the relationship between exposure to DON-contaminated diet and concentration of lysozyme in skin mucus. No effect of the mycotoxin-contaminated diet on lysozyme concentration was observed. Lysozyme activity in the skin mucus of gilthead seabream *Sparus aurata* showed no significant correlation with other immune substances, which suggests that lysozyme is constitutively secreted in the skin mucus irrespective of other humoral immune substances in this species (Jung et al. 2012).

The results of the study indicate that exposure to deoxynivalenol at 1964 μg/kg feed induces changes in pro-inflammatory cytokines and thus may influence the immune responses of rainbow trout. This could be useful for providing a better understanding of the toxicological effect of the mycotoxin deoxynivalenol on cultured fish such as rainbow trout, an important species in commercial aquaculture.

REFERENCES

Balfry SK, Iwama GK (2014): Observations on the inherent variability of measuring lysozyme activity in coho salmon (Oncorhynchus kisutch). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 138, 207–211.

Bracarense AP, Lucioli J, Grenier B, Drociunas Pacheco G, Moll WD, Schatzmayr G, Oswald IP (2012): Chronic ingestion of deoxynivalenol and fumonisin, alone or in interaction, induces morphological and immunological changes in the intestine of piglets. British Journal of Nutrition 107, 1776–1786.

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). Toxicology and Applied Pharmacology 193, 188–201.

Esteban MA (2012): An Overview of the immunological defences in fish skin. ISRN Immunology, doi: 10.5402/2012/853470.

Fink-Gremmels J (2008): Mycotoxins in cattle feeds and carry-over to dairy milk: A review. Food Additive and Contaminants Part A 25, 172–180.

Guan S, He J, Young JCH, Zhu H, Li XZ, Ji CH, Zhou T (2009): Transformation of trichothecene mycotoxins by microorganisms from fish digesta. Aquaculture 290, 290–295.

Hooft JM, Elmor AEHI, Encarnacao P, Bureau DP (2011): Rainbow trout (Oncorhynchus mykiss) is extremely sensitive to the fed-borne Fusarium mycotoxin deoxynivalenol (DON). Aquaculture, 311, 224–232.

Jung TS, Del Castilo CS, Javaregowda PK, Dalvi RS, Nho SW, Park SB, Jang HB, Cha IS, Sung HW, Hikima J, Aoki T (2012): Seasonal variation and comparative analysis of non-specific humoral immune substances in the skin mucus of olive flounder (Paralichthys olivaceus). Developmental and Comparative Immunology 38, 295–301.

Manning BB, Abbas HK (2012): The effect of Fusarium mycotoxins deoxynivalenol, fumonisin, and moniliformin from contaminated moldy grains on aquaculture fish. Toxin Reviews 31, 11–15.

Matejova I, Modra H, Blahova J, Franc A, Fictum P, Sevcikova M, Svobodova Z (2014): The effect of mycotoxin

deoxynivalenol on haematological and biochemical indicators and histopathological changes in rainbow trout (Oncorhynchus mykiss). Biomed Research International 2014; 310680.

Ondrackova M, Valova Z, Kortan J, Vojtek L, Adamek Z (2012): Consequent effects of the great cormorant (Phalacrocorax carbo sinensis) predation on parasite infection and body condition of common carp (Cyprinus carpio). Parasitology Research 110, 1487–1493.

Perez-Sanchez T, Balcazar JL, Merrifield DL, Carnevali O, Gioacchini G, de Blas I, Ruiz-Zarzuela I (2011): Expression of immune-related genes in rainbow trout (Oncorhynchus mykiss) induced by probiotic bacteria during Lactococcus garvieae infection. Fish and Shellfish Immunology 31, 196–201.

Pestka JJ (2010): Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Archives of Toxicology 84, 663–679.

Pestka JJ, Zhou HR, Moon Y, Chung YJ (2004): Cellular and molecular mechanisms for immune modulation by deoxynivalenol and other trichothecenes: unraveling a paradox. Toxicology Letters 153, 61–73.

Pietsch C, Kersten S, Burkhardt-Holm P, Valenta H, Danicke S (2013): Occurence of deoxynivalenol and zearalenone in commercial fish feed: an initial study. Toxins 5, 184–192.

Pietsch C, Michel C, Kersten S, Valenta H, Dänicke S, Schulz C, Kloas W, Burkhardt-Holm P (2014): In vivo effects of deoxynivalenol (DON) on innate immune responses of carp (Cyprinus carpio L.). Food and Chemical Toxicology 68, 44–52.

Pinton P, Tsybulskyy D, Lucioli J, Laffitte J, Callu P, Lyazhri F, Grosjean F, Bracarense AP, Kolf-Clauw M, Oswald IP (2012): Toxicity of deoxynivalenol and its acetylated derivatives on the intestine: differential effects on morphology, barrier function, tight junction proteins, and mitogen-activated protein kinases. Toxicological Sciences 130, 180–190.

Poisot T, Simkova A, Hyrsl P, Morand S (2009): Interaction between immunocompetence, somatic condition and parasitism in the chub Leuciscus cephalus in early spring. Journal of Fish Biology 75, 1667–1682.

Press CM, Evensen Ø (1999): The morphology of the immune system in teleost fishes. Fish and Shellfish Immunology 9, 309–318.

Sanden M, Jorgensen S, Hemre GI, Ornsrud R, Sissener NH (2012): Zebrafish (Danio rerio) as a model for investigating dietary toxic effects of deoxynivalenol contamination in aquaculture feeds. Food and Chemical Toxicology 50, 4441–4448.

Secombes C (2008): Will advances in fish immunology change vaccination strategies? Fish and Shellfish Immunology 25, 409–416.

Streit E, Schatzmayr G, Tassis P, Tzika E, Marin D, Taranu I, Tabuc C, Nicolau A, Aprodu I, Puel O, Oswald IP (2012): Current situation of mycotoxin contamination and co-occurrence in animal feed-focus on Europe. Toxins 4, 788–809.

Sugita-Konishi Y, Park BJ, Kobayashi-Hattori K, Tanaka T, Chonan T, Yoshikawa K, Kumagai S (2006): Effect of cooking process on the deoxynivalenol content and its subsequent cytotoxicity in wheat products. Bioscience, Biotechnology and Biochemistry 70, 1764–1768.

Vandenbroucke V, Croubels S, Martel A, Verbrugghe E, Goossens J, Van Deun K, Boyen F, Thompson A, Shearer N, De Backer P, Haesebrouck F, Pasmans F (2011): The mycotoxin deoxynivalenol potentiates intestinal inflammation by Salmonella typhimurium in porcine ileal loops. PLoS One 6, e23871.

Wache YJ, Valat C, Postollec G, Bougeard S, Burel C, Oswald IP, Fravalo P (2009): Impact of deoxynivalenol on the intestinal microflora of pigs. International Journal of Molecular Science 10, 1–17.

Wegulo SN (2012): Factors influencing deoxynivalenol accumulation in small grain cereals. Toxins 4, 1157–1180.

Woodward B, Young LG, Lun AK (1983): Vomitoxin in diets for rainbow trout (Salmo gairdneri). Aquaculture 35, 93–101.

Zelnickova P, Matiasovic J, Pavlova B, Kudlackova H, Kovaru F, Faldyna M (2008): Quantitative nitric oxide production by rat, bovine and porcine macrophages. Nitric Oxide 19, 36–41.

Corresponding Author:

Iveta Matejova, University of Veterinary and Pharmaceutical Sciences, Palackeho 1/3, 612 42 Brno, Czech Republic
E-mail: H15001@vfu.cz