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Fusarium mycotoxin-contaminated wheat containing deoxynivalenol alters the gene expression in the liver and the jejunum of broilers

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The effects of mycotoxins in the production of animal feed were investigated using broiler chickens. For the feeding trial, naturally Fusarium *mycotoxin-contaminated wheat was used, which mainly contained deoxynivalenol (DON). The main effects of DON are reduction of the feed intake and reduced weight gain of broilers. At the molecular level, DON binds to the 60 S ribosomal subunit and subsequently inhibits protein synthesis at the translational level. However, little is known about other effects of DON, for example, at the transcriptional level. Therefore, a microarray analysis was performed, which allows the investigation of thousands of transcripts in one experiment. In the experiment, 20 broilers were separated into four groups of five broilers each at day 1 after hatching. The diets consisted of a control diet and three diets with calculated, moderate concentrations of 1.0, 2.5 and 5.0 mg DON/kg feed, which was attained by exchanging uncontaminated wheat with naturally mycotoxin-contaminated wheat up to the intended DON concentration. The broilers were held at standard conditions for 23 days. Three microarrays were used per group to determine the significant alterations of the gene expression in the liver ($P < 0.05$), and qPCR was performed on the liver and the jejunum to verify the results. No significant difference in BW, feed intake or feed conversion rate was observed. The nutrient uptake into the hepatic and jejunal cells seemed to be influenced by genes:* SLC2A5 *(fc: −1.54, DON2.5), which facilitates glucose and fructose transport and* SLC7A10 *(fc: +1.49, DON5), a transporter of* D*-serine and other neutral amino acids. In the jejunum, the palmitate transport might be altered by* SLC27A4 *(fc: −1.87, DON5) and monocarboxylates uptake by* SLC16A1 *(fc: −1.47, DON5). The alterations of the SLC gene expression may explain the reduced weight gain of broilers chronically exposed to DON-contaminated wheat. The decreased expressions of* EIF2AK3 *(fc: −1.29, DON2.5/5) and* DNAJC3 *(fc: −1.44, DON2.5) seem to be related to the translation inhibition. The binding of DON to the 60 S ribosomal subunit and the subsequent translation inhibition might be counterbalanced by the downregulation of* EIF2AK3 *and* DNAJC3*. The genes* PARP1, MPG, EME1, XPAC, RIF1 *and* CHAF1B *are mainly related to single-strand DNA modifications and showed an increased expression in the group with 5 mg DON/kg feed. The results indicate that significantly altered gene expression was already occurring at 2.5 mg DON/kg feed.*

Keywords: deoxynivalenol, liver, broiler, gene expression, microarray

Implications

The mycotoxin deoxynivalenol (DON) is a frequently detected compound produced by moulds, which grow on wheat and other cereals during pre-harvest. The main effects of DON in broilers are reduced feed consumption and weight gain. The measurement of the gene expression in the liver gave indications on the functional mechanism of DON. Genes related to altered nutrient uptake to the cell, detoxification, altered protein synthesis rate and DNA repair mechanisms could be detected. With the description of new pathways, a better understanding of the effects of DON on the metabolism of broiler chicks can be expected.

Introduction

About 25% of the world's food crops are contaminated with mycotoxins. The most frequently occurring and widespread mycotoxin in feedstuffs for poultry in European countries is deoxynivalenol (DON) (Awad *et al.*, 2004). The mycotoxin-contaminated wheat used in this study contained mainly

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DON, in addition to a very low contamination with T-2 toxin and zearalenone (ZON) in relation to maximal guidance values in the European Union (EU). DON is a type B trichothecene, a secondary metabolite produced by *Fusarium* species mainly from *Fusarium graminearum* and *Fusarium culmorum*. Owing to the growth of the fungi on maize, wheat and other cereals, poultry feed can contain DON (Rotter *et al*., 1996; Awad *et al*., 2004). In the EU, the maximal permitted value for feed contamination is 5 mg DON/kg feed in poultry nutrition. At that level, no damage to the birds is expected (European Union, 2006). However, decreased feed intake, weight gain and reduced peripheral blood monocytes have been observed in broilers fed 9.3 mg/kg DON in the grower phase (Swamy *et al*., 2004). DON fed to pigs either in a purified form or in the form of naturally contaminated corn led to reduced weight gain and feed intake. Until the end of the experiment, the animals receiving the purified DON recovered from the growth depression in contrast to the animals receiving naturally contaminated corn. The differences between naturally DON-contaminated feed sources and the feed spiked with purified DON might be caused by fungal components present in the naturally mycotoxin-contaminated feed (Prelusky *et al*., 1994). We decided to use naturally mycotoxin-contaminated wheat for the experiment to reproduce the effects that occur in the commercial broiler production. It was suggested that the DON tolerance of broilers results from poorer toxin bioavailability and rapid elimination from the body. DON is rapidly absorbed in the upper part of the gastrointestinal tract and is also quickly cleared from the body of the chickens. It has been suggested that an efficient clearance takes place due to a hepatic or renal first-pass effect in chickens. The oral administration of ^{14}C-DON to chickens showed high radioactivity in the liver and bile, with more than 90% of the original label occurring in the excreta before 48 h (Awad *et al*., 2008). Another experiment showed that in hens administered ^{3}H-DON, an overwhelming amount of radioactivity was detected in the urine, demonstrating the absorption of DON by the digestive system (Lun *et al*., 1989). The main DON metabolite detected in urine and faeces was de-epoxy DON (DOM-1) (Yoshizawa *et al*., 1986). Therefore, the liver, as a direct target of DON, was chosen to analyse the gene expression with microarrays. It has been suggested that cells and tissues with high protein turnover rate, such as the liver and small intestine, are most affected by DON (Awad *et al*., 2008). It was also observed that fast-growing broilers are more susceptible to DON than laying hens (Rotter *et al*., 1996). Pestka (2007) claimed that DON is metabolised in all species, and that it does not accumulate. The main effect of DON is the binding to the 60 S ribosomal subunit and the subsequent prevention of polypeptide chain initiation or elongation (Ueno, 1984). Further, DON affects the immune system either as an immunosuppressive or immunostimulative, depending on the dose and exposure regime. The immunosuppression was explained mainly by the binding capacity of DON to the ribosomes and by the inhibition of protein synthesis (Bondy and Pestka, 2000). However, there is little or no information available about the involvement of DON in the pathways of immune suppression, nutritional uptake or detoxification within the living animal. Most of the studies at the cellular level focused on the acute effects of DON administration. Long-term effects of DON-contaminated cereals seem to be important for animal production because of a regular occurrence of DON-contaminated feed.

The microarrays used in our experiment contained 37 000 probe sets corresponding to more than 28 000 genes. Especially when little is known about the molecular effects of a substance, the use of microarrays as a screening method provides an invaluable starting point. The sequence of the probe sets was designed according to the sequence information from all transcribed genes, including the ones with an unknown function. The profiling of gene expression with microarrays should help gain insight into the effects and metabolism-related genes upon DON administration. In addition, the expression of genes, with a biological relevance to DON administration, was verified with reverse transcription (RT) qPCR in the liver and the jejunum, to cover the main target organs of DON. Possible marker genes and affected pathways should be identified to achieve a better understanding of how chronic and low doses of mycotoxin-contaminated feed uptake affects liver and the small intestine cells in broilers. In addition, the concentration at which DON influences the gene expression in the liver should be reviewed.

Material and methods

Experimental design, birds and diets

Twenty 1-day-old male ROSS 308 broiler chicks were obtained from the commercial hatchery Erb Brüterei AG (Oberdiessbach, Switzerland). The birds were weighed at the beginning of the experiment and separated into four treatment groups according to similar group weight. Five broilers per treatment group per cage were raised together for 23 days. A standard diet (Table 1) was formulated according to the National Research Council (1994) and prepared for all groups in the same process. DON (52 mg/kg) was added to the basic feed mixture of the treatment groups in the form of mycotoxin-contaminated wheat. DON was determined in the diets using LC–MS/MS; the other mycotoxins were determined in the contaminated wheat because of their low concentration, which was close to or below the detection level in the diet. T-2 toxin and ZON concentrations of the contaminated wheat were determined using LC–MS/MS; ochratoxin A, fumonisin B1 and B2 and aflatoxin B1, B2, G1 and G2 were determined with HPLC-fluorescence derivatisation in an external laboratory (UFAG; Sursee, Switzerland). The pelleted feed and water were provided *ad libitum* from day 1 onwards. The animal experiment was performed in accordance with the regulations of the cantonal veterinary office in Zurich.

The total cage weight and feed intake were monitored on a weekly basis. Total water intake per cage was recorded for 4 days a week. Feed samples were taken from the principal components, the control and the treatment diet before and after pelleting and on days 1 and 23 of the experiment. All the feed samples were stored in a 4°C cooler. Excreta samples were taken from days 8 to 11 and from days 15 to 18

Table 1 *Broiler diets*

	Control	DON1	DON2.5	DON5
DON (mg/kg)	0	1	2.5	5
Ingredients (%)				
Corn		17.5		
Soybean meal 48%		25.0		
Potato protein		2.5		
Rapeseed oil		5.0		
Salt		0.15		
DL-methionine		0.26		
Limestone grit		0.90		
Lysine-HCL		0.17		
DCP 38/40		1.20		
Na-bicarbonate		0.30		
Celite 545		1.52		
Premix[1]		0.50		
Contaminated wheat	0	1.92	4.80	9.62
Uncontaminated wheat	45.00	43.08	40.19	35.39
Analyzed parameters				
DON (mg/kg)	0.32	0.88	2.21	4.42
T-2 toxin (μg/kg)	0	1.0	2.5	5.1
ZON (μg/kg)	0	6.7	16.8	33.7
Dry matter (%)	90.9	90.9	91.2	91.2
G.E. (MJ/kg)	15.85	15.85	15.94	15.74
Crude fat (%)	6.81	6.97	7.03	6.97
Crude protein (%)	20.76	20.73	20.86	20.88
Ash (%)	6.47	6.57	6.70	6.66

DON = deoxynivalenol; DCP = di-basic calcium phosphate; ZON = zearalenone; G.E. = gross energy.
Contaminated wheat (52 mg DON/kg), uncontaminated wheat (0.58 mg DON/kg). Maximal allowed contamination per kg feed of poultry in European Union (2006; percentage in group DON5 of maximal allowed concentration): DON: 5 mg/kg (88.5%), T-2: 150 μg/kg (3.4%), ZON: no recommendations. Mycotoxins below the detection limits: aflatoxin B1, B2, G1, G2 (1.3 μg/kg feed), ochratoxin A (1 μg/kg feed) and fumonisin B1, B2 (10 μg/kg feed).
[1]Premix: diet from Aeschbacher *et al.* (2005).

from all four cages and stored at −20°C. Dry matter, ash, gross energy, crude fat and crude protein content were determined in all feed and excreta samples (Table 1). Apparent metabolisable energy intakes (AME) and nitrogen (N) retention were calculated according to the method described by Scott and Hall (1998). After 23 days, broilers were weighed individually and slaughtered by cervical dislocation. Liver samples were removed immediately and washed, and from the middle portion of the left liver lobe, a slice of about 1 cm width was cut and frozen in liquid N. In a similar way, a slice of the jejunum was collected; first 7 cm below the angle of Treitz, a 4-cm-long piece from the jejunum was cut out, and then the lumen was opened and rinsed with 0.9% NaCl before it was also frozen in liquid N.

High-density oligonucleotide array hybridisation

Total RNA was isolated from 12 frozen liver samples with TRIZOL reagent (Invitrogen, Basel, Switzerland). From all four groups, three samples were selected according to the weight of the birds. The heaviest and lightest birds were excluded. The samples were homogenised in a mortar filled with liquid N. After isolation, RNA concentration and purity were determined with an ND-1000 (NanoDrop Technologies, Wilmington, DE, USA) and further cleaned up with the RNeasy Mini Kit (Qiagen, Hilden, Germany). Before RT, the RNA integrity was determined using a Bioanalyzer 2100 (Agilent Technologies, Basel, Switzerland). The integrity number was calculated using the Bioanalyzer 2100 software with a value between 1 (for totally degraded RNA) and 10 (for undegraded RNA). For the microarray experiments, only samples with a value higher than 9 were taken. Labelled cRNA probes were generated according to the protocol from Affymetrix using the GeneChip One-Cycle cDNA Synthesis Kit (Affymetrix, Santa Clara, CA, USA). The procedure consisted of RT of 7 μg total RNA with a T7 oligo (dT) promoter primer and a second-strand cDNA synthesis, followed by an *in vitro* transcription to biotin-labelled cRNA. Finally, the labelled cRNA was fragmented and hybridised at 45°C for 16 h on the Chicken Genome Arrays (Affymetrix, Santa Clara, CA, USA). For every sample a single microarray was used. The arrays were washed and stained using the GeneChip Fluidics Station and scanned with a GeneChip Scanner 3000 according to the manufacturer's protocol (Affymetrix, Santa Clara, CA, USA). The GeneChip operating software (GCOS, Affymetrix, Santa Clara, CA, USA) controlled the scanner and the fluidics station. The data were analysed with the Microarray Suite version 5.0 (MAS 5.0) using Affymetrix default analysis settings and global scaling as a normalisation method. The image data on each individual chip were scaled to target intensity 500.

RT and qPCR

To verify the results from the microarray experiment, the total RNA was extracted from the remaining liver and the jejunum samples. RNA extraction, quantification and quality control were performed in the same manner as the microarray samples. The experiments for 20 samples per tissue were conducted in two steps: first, the RT was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Rotkreuz, Switzerland) and then the real-time PCR (qPCR) was conducted with adjusted amounts of cDNA. The genes for the qPCR were selected after the gene expression analysis and the literature review, depending on their possible biological relevance in relation to mycotoxin-contaminated feed. Primers were designed with Primer Express 3.0 software (Applied Biosystems, Rotkreuz, Switzerland) on two adjoining exons in order to exclude a possible amplification due to genomic DNA contamination. The two endogenous controls *mitochondrial ribosomal protein S18A* (*MRPS18A*) and *ribosomal protein S17* (*RPS17*) turned out to perform the best for adjusting the cDNA concentration, compared with the traditional and other selected control genes such as *GAPDH*, *β-actin*, *DHRS7*, *RPS28*, *APOB* and *LDHA*. Genes and the corresponding primer pairs are indicated in Table 2. The 20-μl qPCR reaction contained 500 nM of forward and reverse primers, 100 ng of sample cDNA and 10 μl Fast SYBR Master Mix (2X; Applied Biosystems, Rotkreuz, Switzerland). The PCR amplification was performed on the 7500 Fast real-time PCR System with SDS Software 1.3.1 (Applied Biosystems,

Table 2 *Primer sequence for qPCR*

Gene	Forward	Reverse
MRPS18A	GGGAAGCGGCTGGGTTT	ATTATGGTCGTGTTGCCTTCAGT
RPS17	TGGATGCGCTTCATCAAGTG	TCATCCCCCAGCAAGAAAGT
AKR1B1	TTAAAGAGATTGCAGCCAAGCA	TCACGTTTCTCTGGATGTGGAA
CASP1	GCAGGAGATGTTCCGAAAGG	ACTTCTTCAGCATTGTAGTCCTCTCTT
EIF2AK3	CCTGTTCTGCCTCATCGTCAT	ACTCAGGGAAGGTGAAGTACATCTG
EP300	GGCTGAGCTGTTGGCATAGC	TCAACCATGAATTCCCAAAACTC
EXOSC9	CGCGTTCCTCTCGTTCATTT	CCTATTTGTGTCAGTTTTGCCTTCT
IFT57	AGGATTAGTGGGCAGTGCAAA	GCTGAAGCTGCTGAGCTACGA
LAPTM4B	CAGGACTACCTTCGCCAGCT	TTCCTTGTAAGGAAAGTTGCTGG
MIA2	GGAATGTGAGACGGCAATGA	TCCCCTGTTTTGAAGCTCAGA
MAPKAPK3	TGGTCTCTGGGTGTCATCACA	AAATAGCTTGTCCAGTGTTCGAGTAG
TP53I3	TGGCCAAGCTGAATATGTTACAGTA	GCTGCAGCCTGAATAAAAGTCA
SLC2A5	ACTGCAGCAACAATGGAGAAGA	GGTGGGACCTCTGGTCAACA
SLC7A10	TGTAAATATATCCTGAATGCGTGTTG	CTAGGATTCTCTCCATGGTGTGTTT
SLC16A1	GCAGGGTTCAGGTTAAATGCA	CACCGTGGAGGAGCTCTACTTC
SLC27A4	TCCTGCCTTCCCCTCAATG	CGCATCCTCAACCTGACAGA
STK39	ACTCGTAACAAAGTAAGGAAAACATTTG	CCTCGCACCTGCTCCATTA
TJP1	TCTTGCTGTGGCTACAACAGTGT	AATGCTGTGCCTAAAGCCATTC
XRN1	ACTTCACTGAAGTGCTCACGATCA	GATATTTCTCAAAGCGCTTTAAATTCAG

5′–3′ direction.

Rotkreuz, Switzerland) and repeated four times separately. The cycling programme was 20 s at 95°C and then 40 cycles of the following: denaturation for 3 s at 95°C, annealing and extension at 60°C for 30 s. The results were evaluated with the comparative Ct method ($2^{-\triangle\triangle Ct}$) with the SDS software.

Statistical analysis

The alterations in the expression profiles between the control and the treatment groups were analysed using the GeneSpring 7.3 software (Agilent Technologies, Basel, Switzerland). Normalisation was carried out with GeneSpring 7.3 and the default settings as follows: values below 0.01 were set to 0.01; each measurement was divided by the 50th percentile of all measurements in that sample and each gene was divided by the 50th percentile of its measurements in all the samples. The prefiltering was performed according to Pepper *et al.* (2007) as follows: the genes had to be present or marginal in all samples, and the raw value had to be above the 50th percentile, of all present and marginal genes, in all samples of a group to remove genes with a low signal strength. The gene lists from the four groups were merged together, and Student's *t*-test was performed between the control and each treatment group ($P < 0.05$). Further, genes were grouped into seven subgroups: first, genes with significant changed expression in only one group (DON1, DON2.5, DON5) compared with the control group; then, genes within two groups (DON1/2.5, DON2.5/5, DON1/5) compared with the control group and genes within all three groups (DON1/2.5/5) compared with the control. Finally, for every subgroup a *t*-test was performed and the *P*-values are indicated in Tables 4 and 5. The fold changes were listed for every comparison. The final gene list was separated into groups of up- and downregulated genes. The microarray raw data have been deposited into the Gene Expression Omnibus (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/geo) with accession number GSE25185. Genes with an average raw value above the 80th percentile, of all present and marginal genes, in the group with the significantly changed expression are indicated with an asterisk (Tables 4 and 5). The average raw value was indicated to estimate the importance of a gene in cellular processes. All the other measured parameters were analysed using Student's *t*-test to determine significance ($P < 0.05$).

Results

Diet characterisation and performance of broilers

The DON concentration of the control diet was 0.32 mg/kg feed, which was close to the detection level of 0.2 mg DON/kg. The DON concentration in DON1 was 0.89 mg/kg, 2.21 mg/kg for DON2.5 and 4.43 mg/kg for DON5. The calculated T-2 toxin and ZON concentrations in group DON5 were low, with values of 5.1 and 34.6 μg/kg feed, respectively. The other measured mycotoxin concentrations were below the detection limit. The performance of the broilers within the groups was balanced, and they did not show an abnormal behaviour or other indications due to DON contamination. The progression of growth and the feed intake were within the range of expectation (Table 3). No behavioural or macroscopic pathological alterations were detected in the appearance of liver, stomach, intestine, spleen, heart, muscles and lungs of the broilers receiving the mycotoxin-contaminated feed.

The parameters for the RNA quality and quantity, as well as the microarray quality parameters, were within the requirements. In Tables 4 and 5, a summary of genes with an altered expression are sorted according to the DON groups.

Table 3 *Performance parameters*

	Control	%	DON 1	%	DON 2.5	%	DON 5	%
BW day 22 (g) ± s.e.	1118 ± 36.0		1140 ± 19.8		1201 ± 30.1		1084 ± 80.4	
FI week 3 (g)	807		839		908		771	
FCR (g/g)	1.50		1.53		1.60		1.54	
AME P1 (MJ/kg)/(%)	15.27	100	14.97	98.1	15.44	101.2	14.75	96.6
AME P2 (MJ/kg)/(%)	14.74	100	14.73	99.9	14.89	101.1	13.87	94.1
N retention P1 (%)	67.74	100	64.72	95.5	69.26	102.3	66.47	98.1
N retention P2 (%)	65.08	100	63.64	97.8	63.79	98.0	59.20	91.0

DON = deoxynivalenol; FI = feed intake; FCR = feed conversion rate (g gain/g feed); AME P1/2 = apparent metabolisable energy in MJ/kg dry matter and % relative to the control; P1/2 = period 1 or 2.

The expression of a total of 566 genes was significantly altered in DON groups compared with the control group (supplementary files). The heatmap of the cluster analysis of all significantly altered genes between the control and the DON-containing groups is shown in Figure 1.

The entire list of up- and downregulated genes is available in the section of supplementary files. In the DON groups, 367 genes were upregulated. Of these, 230 genes have been specified in relation to their function or structure, and for 138 genes only the transcript sequence is known. The average expression of 152 upregulated genes, or 41% of the upregulated genes, was above the 80th percentile of the raw value of all genes, which is indicated by an asterisk (*) in the column 'Fold change' (Tables 4 and 5). The fold change of 28 genes was above two. Venn diagrams are presented in the section of supplementary files with the distribution of significantly altered genes between the treatment groups. A summary of discussed genes with an upregulated expression in the DON groups is presented in Table 4.

In Table 5, the most important and significantly downregulated genes are indicated according to the DON groups. Of 199 significantly downregulated genes, the function of 139 genes was known. Sixty genes were unknown or no function could be assigned to them; 14 genes had a fold change higher than two; and 45 genes, or 32% of the downregulated genes, had a raw value above the 80th percentile.

RT and qPCR

The results from the microarray experiment were verified with RT qPCR, and the results are shown in Table 6. The alterations could be confirmed for 9 of the 15 genes in the liver. A total of 10 of 17 genes showed a significantly altered expression in the liver and the jejunum. The correlation between the fold changes of the qPCR experiment and the microarray analysis in group DON5 was 0.75. *XRN1* and *SLC16A1* expression were measured because of their biological relevance. The genes *AKR1B1*, *MIA2*, *SLC2A5*, *SLC7A10* and *LAPTM4B* were significantly regulated in the liver, measured with qPCR and microarrays, as well as in the jejunum tissues. *SLC27A4*, *CASP1* and *EP300* had a significant altered expression in the microarray experiment in the liver and in the qPCR experiment in the jejunum. The alteration of the expression for the genes *EXOSC9*, *TJP1*, *EIF2AK3* and *IFT57* could be confirmed only in the liver by qPCR. *SLC16A1* was not significantly altered in any measurement in the liver, but showed a decreased expression in the jejunum groups. The reduced expression of *XRN1* did not reach significance in the microarray analysis ($P < 0.08$), but was significantly reduced in the qPCR measurement. The expression alteration of the genes *MAPKAPK3*, *STK39* and *TP53I3* could not be confirmed with qPCR in any tissues. Taking the liver and jejunum groups together, 14 of 17 expression alterations could be confirmed with qPCR.

Discussion

The use of naturally mycotoxin-contaminated wheat resulted primarily in a high DON contamination (4.425 mg/kg) and a low calculated contamination with T-2 (5.1 μg/kg) and ZON (33.7 μg/kg) in the final feed mixture of DON5. Because of the low calculated concentrations of T-2 and ZON approximately or below the detection level, they were measured in naturally mycotoxin-contaminated wheat. The other measured mycotoxins in the mycotoxin-contaminated wheat were below the detection limit. The maximal allowed concentration for DON in poultry feed is 5 mg/kg feed and 150 μg/kg feed for T-2. No maximal guidance values exist for ZON (bovine: 500 μg/kg feed), because of the high tolerance of poultry. The maximal permitted mycotoxin concentrations are related to the level at which no damage to the birds is expected (EFSA, 2004). The mycotoxin analysis in the final feed mixture showed, in comparison with the maximal permitted concentration, a proportion of 88.5% of DON, 3.4% of T-2 and 6.7% of ZON (bovine guidance value) in the feed of group DON5. Because of the high proportion of DON and the low amount of T-2 and ZON, we considered the observed alterations at the transcriptional level due to the effects of DON. Most of the reported effects have been investigated mainly in cell cultures or model organisms such as mice. In our experiment, we showed for the first time similar alterations in the gene expression in broilers.

The growth, feed intake and feed conversion rates were within the normal range in our experiment. Although BW and feed intake were numerically the lowest in group DON5 and the highest in group DON2.5, this was statistically not significant. It has been mentioned by Awad *et al.* (2008) that

Table 4 *Upregulated genes in DON group*

Systematic name	Name	Fc	*P*	Description	Function
Upregulated in DON 1, DON 2.5 and DON 5					
Affx.10275.1.S1_at	SAMD12	1.9, 1.9, 2.0	0.033	Sterile alpha motif domain containing 12	Signalling
9157.1.S1_at	COX19	1.4, 1.7, 1.6	0.016	Cytochrome *c* oxidase assembly homologue	Metal transport
Affx.11292.2.S1_s_at	LOC425649	1.9, 1.5, 1.4	0.006	Similar to rhomboid family 1	Epidermal growth factor receptor
3382.1.S1_at	PPIB	1.4, 1.2, 1.4	0.015	Peptidylprolyl isomerase B (cyclophilin B)	Immune system
Affx.1528.1.S1_at	MAPKAPK3	1.5, 1.3, 1.3	0.001	Mitogen-activated protein kinase APK3	p38 MAPK pathway
Upregulated DON 2.5 and DON 5					
Affx.24663.2.S1_s_at	IFT57	2.0, 3.0	0.004	Intraflagellar transport 57 homologue	Regulation of Apoptosis
Affx.4365.1.S1_at	LOC416968	1.9, 2.0	0.012	Similar to presenilin-like protein 4	Peptidase
2529.2.S1_a_at	IL1RL1	1.7, 1.7	0.024	Interleukin 1 receptor-like 1	Innate immune response
9700.2.S1_a_at	TSPAN7	1.4, 1.4	0.017	Tetraspanin 7	Signal transduction
Upregulated in DON 1 and DON 2.5					
Affx.12452.1.S1_s_at	KLF11	2.5, 2.1*	0.015	Kruppel-like factor 11	Neg. regulation of cell proliferation
Upregulated in DON 1 and DON 5					
16554.2.S1_a_at	LAPTM4B	4.2, 3.1	0.013	Lysosomal associated prot. Transmem. 4β	
10204.1.S1_s_at	CASP1	1.8, 2.2	0.006	Caspase 1	Apoptosis/interleukin 1 convert.
11819.1.S1_at	CENPQ	1.6, 1.8	0.032	Centromere protein Q	Centromeric complex
Affx.10735.1.S1_at	MITD1	1.6, 1.7	0.005	Microtubule interact./transp. dom. cont. 1	Protein transport
2952.1.S1_at	CLDN3	1.6, 1.6	0.031	Claudin 3	Leukocyte transendothelial migrat.
Affx.12447.1.S1_at	SLC16A9	2.0, 1.6	0.002	Solute carrier fam. 16, mem. 9	Monocarboxylic acid transporter
3364.2.S1_at	LOC425437	1.7, 1.5	0.025	Similar to phosphatidylinositol glycan B	Glycolipid mannosyltransf. activity
2252.1.S1_s_at	RAB33B	1.5, 1.4	0.018	RAB33B, member RAS oncogene family	Protein transport
Affx.11111.1.S1_s_at	GDPD5	2.1, 1.4	0.017	Glycerophosphodiester phosphodiester. 5	Glycerol metabolic process
Upregulated in DON 5					
12857.1.S1_s_at	TP53I3	2.646	0.025	Tumor protein p53 inducible protein 3	Apoptosis by oxidative stress
Gga.13986.1.S1_at	IFT57	2.617	0.003	Intraflagellar transport 57 homologue	Regulaiton of apoptosis
4124.1.S1_at	LOC395933	2.597*	0.028	Sulfotransferase	Sulfotransferase activity
4332.1.S1_at	HSPCB	1.972	0.030	Heat shock 90kDa protein 1, beta	Response to unfolded protein
635.1.S1_at	GLRX	1.901*	0.031	Glutaredoxin (thioltransferase)	Cell redox homeostasis
Affx.7092.1.S1_at	GNPAT	1.869*	0.029	Glyceronephosphate O-acyl-transferase	Fatty acid metabolic process
Affx.12177.1.S1_s_at	MCM5	1.832	0.022	Minichromosome maintenance complex 5	DNA replication, cell cycle
Affx.8046.1.S1_s_at	SLC41A2	1.832	0.032	Solute carrier family 41, member 2	Cation transport
Affx.24672.1.S1_s_at	PROS1	1.812*	0.011	Protein S (alpha)	Blood coagulation
3251.1.S1_at	NDUFS1	1.802	0.013	NADH dehydrogenase Fe-S protein 1	ATP metabolic process
Affx.12875.1.S1_at	VPS29	1.779	0.037	Vacuolar protein sorting 29 homologue	Protein transport
11184.1.S1_at	KCTD12	1.757	0.016	Potassium channel tetram. dom. cont. 12	Potassium ion transport
7164.1.S1_at	LOC421110	1.724	0.025	Similar to DNA replication initiator protein	DNA replication initiation
5235.1.S1_at	HYOU1	1.695*	0.005	Hypoxia upregulated 1	Response to hypoxia
Affx.5663.1.S1_at	SMAD7	1.684	0.022	SMAD family member 7	Signalling
8338.1.S1_at	NIP7	1.667*	0.047	Nuclear import 7 homologue	Ribosome assembly
Affx.21723.1.S1_at	GPSM1	1.645	0.001	G-protein signalling modulator 1	Cell differentiation

Table 4 *Continued*

Systematic name	Name	Fc	*P*	Description	Function
Affx.4506.2.S1_s_at	RAB3IL1	1.634	0.014	RAB3A interacting protein (rabin3)-like 1	Guanylnucleotide exchange factor
7843.1.S1_at	SYPL1	1.626*	0.016	synaptophysin-like 1	Transport
13290.1.S1_at	RNFT1	1.582*	0.002	Ring finger protein, transmembrane 1	Metal ion binding
11918.1.S1_at	NSUN3	1.582	0.009	NOL1/NOP2/Sun domain family 3	Transferase activity
Affx.12146.1.S1_at	SNRPA1	1.558	0.033	Small nuclear ribonucleoprotein A	RNA splicing
Affx.13032.1.S1_s_at	KIF23	1.550	0.014	Kinesin family member 23	Mitotic spindle elongation
6206.1.S1_s_at	CHMP6	1.536	0.028	Chromatin modifying protein 6	Protein transport
Affx.12982.1.S1_s_at	COPB1	1.536*	0.027	Coatomer protein complex, subunit β 1	Vesicle-mediated transport
Affx.8553.1.S1_s_at	PBX4	1.524	0.042	Pre-B-cell leukaemia homeobox 4	Transcription regulation
Affx.13133.1.S1_s_at	BPGM	1.520	0.038	2,3-bisphosphoglycerate mutase	Glycolysis
Affx.23087.1.S1_at	NUBPL	1.515	0.037	Nucleotide binding protein-like	Chromosome partitioning
8478.1.S1_s_at	OTOA	1.513*	0.029	Otoancorin	Transport
7249.1.S1_a_at	CDT1	1.473	0.024	Chromatin licensing/DNA replication 1	Reg. of DNA replication initiation
Affx.20694.1.S1_s_at	ORMDL1	1.468*	0.013	ORM1-like 1	Protein folding
2136.3.S1_at	RHOU	1.437	0.017	Ras homologue gene family, member U	Signal transduction
4314.1.S1_at	P50	1.435	0.018	Dynamitin	Microtubule-based process
8862.1.S1_s_at	CHAF1B	1.435	0.033	Chromatin assembly factor 1, subunit B	DNA replication
Affx.23168.1.S1_at	ALDH6A1	1.429*	0.042	Aldehyde dehydrogenase 6 family A1	Propanoate metabolism
1737.1.S1_at	SLC27A4	1.427*	0.027	Solute carrier family 27, member 4	Fatty acid transporter
6330.3.A1_at	RNF7	1.425	0.030	Ring finger protein 7	Ubiquitin cycle Apoptosis
4774.1.S1_at	LOC422278	1.422*	0.027	Nonhistone chrom. protein HMG-14A	DNA binding
229.1.S1_at	HINT1	1.414*	0.025	histidine triad nucleotide binding protein 1	Signal transduction
5209.1.S2_at	ADPRT	1.410*	0.005	Poly (ADP-ribose) polymerase family 1	Base excision repair
840.2.S1_a_at	P08296	1.406	0.003	Myosin alkali light chain mRNA	Motor activity
Affx.22246.1.S1_at	MPG	1.406	0.031	N-methylpurine-DNA glycosylase	DNA repair
Affx.12559.1.S1_s_at	SLC10A7	1.399	0.030	Solute carrier family 10, member 7	Sodium/bile acid cotransporter
Affx.21403.1.S1_s_at	EME1	1.366	0.048	Essential meiotic endonuclease 1	DNA repair
2794.1.S1_at	EP300	1.321*	0.029	E1A binding protein p300	Signal transduction
3310.1.S1_at	XPAC	1.279*	0.046	Xeroderma pigmentosum, compl. A	Nucleotide-excision repair
Affx.2936.1.S1_at	SLC7A10	1.263	0.031	Solute carrier family 7, member 10	Neutral amino acid transporter
7684.1.S1_at	MRPL46	1.256*	0.029	Mitochondrial ribosomal protein L46	Ribosomal protein
5561.1.S1_at	RIF1	1.235	0.001	RAP1 interacting factor homologue	Response DNA damage stimulus
Upregulated in DON 2.5					
554.1.S1_at	HGF	2.105	0.003	Hepatocyte growth factor	Growth factor activity
7604.2.S1_a_at	PLSCR1	1.812	0.012	Phospholipid scramblase 1	Phospholipid scrambling
5517.1.S1_s_at	LOC421267	1.799	0.031	Similar to nuclear DNA-binding protein	Apoptosis inducing
Affx.24639.1.S1_at	FILIP1L	1.762	0.035	Filamin A interacting protein 1-like	Cell proliferation
3674.1.S1_at	STK39	1.650	0.001	Serine threonine kinase 39	Response to cellular stress
6223.1.S1_at	ADAM33	1.621	0.014	ADAM metallopeptidase domain 33	Proteolysis
Affx.3622.2.S1_s_at	ABCG2	1.600*	0.031	ATP-binding cassette, sub-family G 2	Response to drug biomarker
Affx.7445.2.S1_s_at	CSTF3	1.560	0.029	Cleavage stimulation factor, 3′ pre-RNA 3	mRNA processing

Table 4 *Continued*

Systematic name	Name	Fc	*P*	Description	Function
16921.1.S1_at	Tcp11	1.558*	0.042	t-complex 11 (mouse)-like 2	Multicellular organismal develp.
2439.1.S1_at	TMED10	1.511*	0.018	Transmembrane emp24-like trafficking10	Vesicle-mediated transport
Affx.26112.1.S1_at	FUCA1	1.511	0.016	Fucosidase, alpha-L-1	N-Glycan degradation
Affx.6854.2.S1_s_at	C8A	1.431*	0.016	Complement component 8, alpha	Complement activation
Affx.5975.1.S1_at	RAD51L1	1.395	0.028	RAD51-like 1 (*Saccharomyces cerevisiae*)	DNA repair

DON = deoxynivalenol; Fc = fold change of groups DON1, DON2.5 and DON5; *P* = *P*-value of Student's *t*-test.
The systematic name of the probe sets in chicken is the species code Gga., followed by the identifier (e.g. GgaAffx.5975.1.S1_at or Gga.2439.1.S1_at).
*Raw value above 80th percentile.

moderately high concentration levels of DON can even have growth-promoting effects. Further, the increased s.e. in BW of group DON5 indicates that different sensitivity towards DON-contaminated feed might exist, depending on the genetic background of the animals.

Nutrient transport

The gene expression analysis has shown that the nutrient uptake into the cell seems to be influenced by the mycotoxin-contaminated feed. The passive D-fructose transporter with a very low glucose affinity, SLC2A5 (fc: −1.259, DON2.5), had a decreased expression in the liver (fc: −1.54, DON2.5) and the jejunum (fc: −1.94, DON2.5/5). The numerical decrease in AME by 5.9% might be a further indication of a reduced monosaccharide uptake in the jejunum. Maresca *et al.* (2002) showed in their study that fructose uptake into the cell and corresponding transporter SLC2A5 were inhibited by 42% in the intestinal cell line HT-29-D4 cells because of DON administration. Our findings indicate that similar effects occur in the jejunum of broilers in the living animal, and to a lesser extent in the liver. The gene *SLC27A4* (fc: +1.427, DON5) is a palmitate transporter and the increased expression could not be confirmed with qPCR in the liver. However, the expression was significantly reduced in the jejunum (fc: −1.87, DON5). In primary enterocytes treated with *SLC27A4* antisense, palmitate and oleate uptake were reduced simultaneously (Stahl *et al.*, 1999). Maresca *et al.* (2002) reported an increased palmitate uptake by 35% in their study after challenging HT-29-D4 cells with DON. We assume that chronic exposure to DON can invert the regulation or that another functional mechanism on the transport activity is responsible for the discrepancy between the decreased expression of *SLC27A4* in the jejunum and the increased palmitate uptake reported by Maresca *et al.* (2002). The SLC7A10 protein (fc: +1.26, DON5) transports D-serine, glycine and L-isomers of alanine, serine, threonine and cysteine (Nakauchi *et al.*, 2000). The expression change was confirmed with qPCR in the liver (fc: +1.49, DON5) and the jejunum (fc: +2.52, DON5). Further, the N retention was numerically reduced by 9%, which gives an indication on the amino acid uptake. The decrease in L-serine uptake in the cell culture study by Maresca *et al.* (2002) is in disagreement with our observation that *SLC7A10* showed an upregulated expression, but is in agreement with the reduced N retention in group DON5. The discrepancy could indicate a direct inhibition of this L-serine transporter by DON, resulting in the upregulation of SLC7A10. SLC7A5 (fc: −1.218, DON5) is a transporter of neutral amino acids with branched or aromatic side chains such as leucine (Kanai *et al.*, 1998). In an incorporation study with mice, the ^{14}C leucine incorporation into the liver was decreased at 20 mg DON/kg feed. It was assumed that DON inhibited the protein synthesis, and therefore the reduction of the ^{14}C leucine amount occurred (Robbana-Barnat *et al.*, 1987). The addition of 0.5 and 5 μg DON/ml to Caco-2 cells led to a decreased [^{3}H] leucine incorporation and a parallel decrease of cellular protein content (De Walle *et al.*, 2010). The extent to which leucine uptake into the cell is responsible for

Table 5 *Downregulated genes in DON groups*

Systematic name	Name	Fc	*P*	Description	Function
Downregulated in DON 1, DON 2.5 and DON 5					
19878.1.S1_at	ARID1A	1.7, 1.7, 1.6	0.006	AT rich interactive domain 1A	Andro./estrogen receptor signalling
14829.1.S1_at	Mlh3	1.2, 1.3, 1.2	0.006	Sim. to DNA repair protein Mlh3	DNA repair
Downregulated in DON 2.5 and DON 5					
Affx.26.1.S1_at	LOC427047	1.6, 2.1	0.021	Sim. to breast cancer-associated antigen 1	Transcription regulation
Affx.12182.1.S1_at	ODF2	2.0, 1.7	0.01	Outer dense fibre of sperm tails 2	Cell differentiation
Affx.5339.1.S1_at	EXOC4	1.9, 1.7	0.005	Exocyst complex component 4	Protein transport
Affx.25933.3.S1_s_at	PITPNC1	1.7, 1.6	0.006	Phosphatidylinositol transfer protein 1	Lipid transport
15747.2.S1_s_at	APRIN	1.6, 1.5	0.017	Androgen-induced proliferation inhibitor	Cell cycle
Downregulated in DON 1 and DON 2.5					
6220.5.S1_a_at	ICER	1.6, 2.0	0.006	ICER protein	Transcription regulation
Affx.25502.2.S1_at	SLC2A5	1.6, 1.3*	0.004	Solute carrier family 2, member 5	Glucose/fructose transport
Downregulated in DON 5					
9580.2.S1_a_at	AKR1B1	3.948*	0.047	Aldo-keto reductase family 1, member B1	Response to stress
12430.1.S1_at	MIA2	2.544	0.013	Melanoma inhibitory activity 2	Inflammation
Affx.6850.1.S1_at	BTBD7	1.769	0.001	BTB (POZ) domain containing 7	Related to tumourigenesis
14891.1.S1_s_at	RBBP6	1.651	0.039	Retinoblastoma binding protein 6	Protein ubiquitination
9934.1.S1_s_at	ANKRD13A	1.631	0.037	Ankyrin repeat domain 13A	Probably DNA damage related
13135.2.S1_a_at	ZCRB1	1.586	0.023	Zinc finger CCHC-type, RNA binding 1	RNA splicing
9603.1.S1_s_at	USP8	1.529	0.021	Ubiquitin specific peptidase 8	Cell proliferation
12626.2.S1_s_at	HCFC2	1.524	0.003	Host cell factor C2	Transcription regulation
Affx.26072.2.S1_s_at	TJP1	1.52	0.025	Tight junction protein 1 (zona occludens 1)	Intercellular junction assembly
3068.1.S1_at	Trap150	1.511*	0.028	Sim. to thyroid hormone receptor-associ.	Androgen receptor signalling
Affx.20095.1.S1_at	EXOSC9	1.503	0.010	Exosome component 9	Immune response
Affx.26106.6.S1_s_at	LOC428354	1.48	0.036	Sim. to Jumonji domain cont. protein 2B	Transcription regulation
16149.1.S1_s_at	ANKMY1	1.474	0.024	Ankyrin repeat and MYND domain 1	Protein binding
Affx.7493.1.S1_s_at	LOC422658	1.473*	0.037	Sim. to UDP-glucuronosyltransferase 2A1	Detection of chemical stimulus
Affx.8082.2.A1_at	ELOVL2	1.472*	0.030	Elongation of very long chain fatty acid L2	Fatty acid biosynthesis
Affx.21354.1.S1_at	YPEL1	1.438*	0.039	Yippee-like 1 (Drosophila)	Cell morphology
Affx.13221.1.S1_s_at	MTPN	1.425	0.003	Myotrophin	Cell growth
11668.1.S1_at	NDRG1	1.395*	0.029	N-myc downstream regulated gene 1	Response to metal ion
Affx.12896.1.S1_at	SLC7A5	1.218	0.003	Solute carrier family 7, A5	Cationic amino acid transporter
Downregulated in DON 2.5					
12243.1.S1_at	RWDD2B	1.891	0.023	RWD domain containing 2B	Unknown
10529.1.S1_at	LOC770294	1.702	0.008	Similar to RNase H, putative	DNA integration
6038.1.S1_at	LOC417856	1.649	0.045	Sim. to TMEM19 protein	Multi-pass membrane protein
8763.1.S1_at	YTHDC1	1.565	0.025	YTH domain containing 1	RNA splicing
Affx.21020.1.S1_s_at	BAZ1B	1.541	0.035	Bromodomain adjacent to zinc finger 1B	Chromatin remodelling
Affx.13074.1.S1_at	DNAJC3	1.435	0.040	DnaJ (Hsp40) homologue, sub-family C3	Defense response
10975.1.S1_s_at	ARID1B	1.407	0.040	Sim. to AT rich interactive dom. 1B, 1	Chromatin-med. transcription
Affx.10171.1.S1_at	EIF2AK3	1.286	0.010	Eukary. Transl. initiation fact. 2-α kinase 3	Negative regulation of translation

DON = deoxynivalenol; Fc = fold change of groups DON1, DON2.5 and DON5; *P* = *P*-value of Student's *t*-test.
The systematic name of the probe sets in chicken is the species code Gga., followed by the identifier (e.g. GgaAffx.5975.1.S1_at or Gga.2439.1.S1_at).
*Raw value above the 80th percentile.

the decrease of the protein synthesis remains unclear. In a related, unpublished study, *SLC16A1* expression was significantly altered, and therefore the test was also used to verify the expression in the jejunum in this study. *SLC16A1* (fc: −1.87, DON5) had a decreased expression in the jejunum, and it transports monocarboxylates such as butyrate, lactate and pyruvate (Lambert *et al*., 2002). It has been suggested that effects of DON on membrane transport is a result of specific modulation of the transporters and is not due to cell damage (Maresca *et al*., 2002), which is supported by our observations, especially because *SLC7A10* was upregulated. The alteration of these five gene expression levels influences the nutrient uptake into the cell and might have implications for the growth rate of birds.

Detoxification

AKR1B1 (fc: −3.511, DON2.5 and 5) had a decreased expression in the liver, measured with microarray and qPCR experiments (fc: −2.5, DON2.5 and 5), as well as in the jejunum (fc: −2.49, DON5). AKR1B1 reduces reactive aldehydes and ketones to primary and secondary alcohols. Reactive oxygen species also target polyunsaturated fatty acids that form lipid

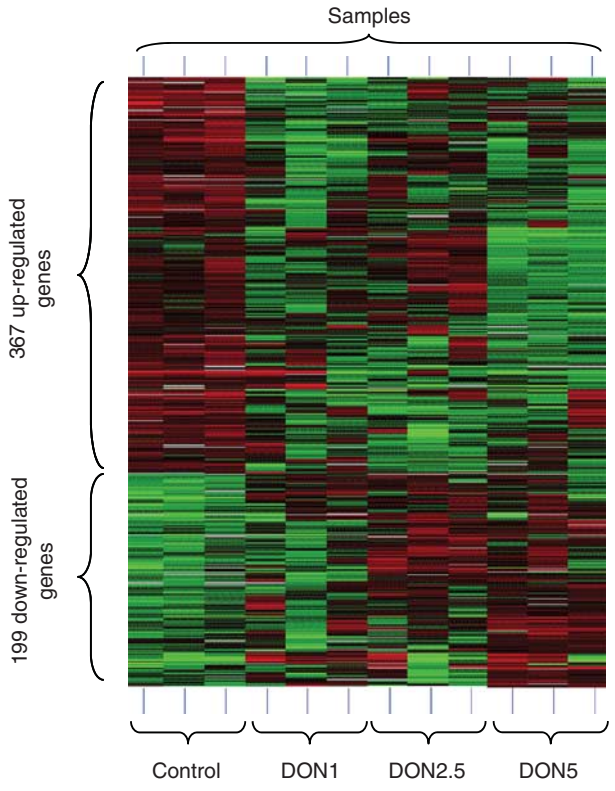

Figure 1 Heatmap of cluster analysis. Heatmap: green, upregulated genes; black, normal expression; red, down-regulated genes. The regulation is displayed according to significant alterations between control group and DON-containing groups. Each horizontal line corresponds to a normalised expression of a gene from the respective sample.

hydroperoxides, which are finally decomposed to form reactive lipid aldehydes, which are highly mutagenic. AKR1B1 detoxifies those lipid aldehydes that can react with bases in DNA to form etheno- and heptano-etheno-DNA adducts (Jin and Penning, 2007). The downregulation of *AKR1B1* by chronic DON exposure might also be in relation to several upregulated DNA repair genes, which are discussed in the section below. In the human hepatoma-derived cell line HepG2, the expression of *AKR1B1* was significantly reduced after the cells were challenged with a series of cytotoxic substances (Kawata *et al.*, 2007). In conclusion, *AKR1B1* seems to be an interesting *in vivo* biomarker for DON-induced alteration of gene expression.

DNA repair

Six genes were significantly upregulated in DON5 and one in DON2.5, which are responsible for DNA damage repair and recognition, showing a possible effect of DON on DNA integrity. The six genes correspond to 4.1% of significantly altered genes, with a known function, in group DON5. The protein is involved in DNA repair and has a critical role in signalling DNA single-strand breaks (Plummer and Calvert, 2007). Shifrin and Anderson (1999) showed that the addition of DON to the Jurkat human lymphoid cell line led to the activation of pro-caspase-3, leading to a higher cleavage of PARP1, a substrate of caspase-3. In murine macrophages, J774A.1, an increased amount of PARP1 and significantly increased amount of cleaved PARP1 protein resulted after the challenge with DON (Marzocco *et al.*, 2009). The upregulation of PARP1 therefore shows that similar effects might occur in the liver of broilers. MPG (fc: +1.406, DON5) is responsible for the recognition and excision of alkylated purine bases (O'Brien and Ellenberger, 2004). EME1 (fc: +1.366, DON5) cleaves branched DNA structures. In the absence of EME1, chromosomal aberrations occurred and led to genomic instability (Abraham *et al.*, 2003). The gene *RAD51L1* (fc: +1.395, DON2.5) functions in homologous recombination and DNA double-strand break repair (Miller *et al.*, 2002). CHAF1B (fc: + 1.435, DON5) is part of the complex CAF-1 and the only subunit available on the Chicken Genome Array. The complex is related to nucleotide excision repair and to the repair of single-strand breaks (Mello *et al.*, 2002). RIF1 (fc: +1.235, DON5) functions as a DNA damage response factor (Silverman *et al.*, 2004). XPAC (fc: +1.279, DON5) is involved in nucleotide excision repair (Shimamoto *et al.*, 1995). In male broiler chickens fed with 10 mg DON/kg feed, a comet assay was performed in spleen leukocytes, which detected aberrations, and a significantly higher amount of DNA breaks were found (Frankic *et al.*, 2006). Interestingly, all the DNA repair-related genes mentioned above were only upregulated in group DON5 with the exception of *RAD51L1*, which was upregulated in DON2.5. Genes such as *PARP1*, *MPG*, *EME1*, *XPAC* and *CHAF1B* belong either to the base excision repair or to the nucleotide excision repair pathway. Under reduced activity of AKR1B1, the anchorage of lipid aldehydes to the DNA might have occurred with a higher frequency, and therefore the discussed single-strand repair genes might have shown a higher expression in the group DON5. A single-strand break or modified nucleotides do not lead necessarily to double-strand breaks or, as a result, to detection in the comet assay. Continuing, it seems that the activation of DNA damage-related genes is induced only by a concentration of 4.42 mg DON/kg feed.

Translation initiation

The expression decrease of *EIF2AK3* (fc: −1.286, DON2.5/5) could be confirmed in the liver with qPCR (fc: −1.70). *EIF2AK3* is a target for transcriptional upregulation of the unfolded protein response and phosphorylates eIF2α, a translation initiator (Yang *et al.*, 2000). The phosphorylation of eIF2α inhibits translation initiation and protein synthesis. *EIF2AK3* activation and the subsequent eIF2α phosphorylation prevent the cell from the accumulation of incorrectly folded or unfolded proteins (Ron, 2002). *DNAJC3* (fc: −1.435, DON2.5) is induced during endoplasmic reticulum (ER) stress (van Huizen *et al.*, 2003). *DNAJC3* binds to *EIF2AK3* and disrupts the activity of *EIF2AK3*. Subsequently, eIF2α phosphorylation by *EIF2AK3* is reduced, stimulating the eIF2α-induced protein synthesis (Gale *et al.*, 1998). Our results are consistent with the publication of Yang *et al.* (2000), which showed reduced expression level of *DNAJC3* after 24 h in murine thymoma cell line EL-4 treated with DON. The binding of DON to the 60 S ribosomal subunit and the subsequent translation inhibition might be counterbalanced by

Table 6 *RT qPCR results*

		Liver				Jejunum			
	Microarray	Don 2.5		Don 5		Don 2.5		Don 5	
Gene name	Fc	Fc	*P*	Fc	*P*	Fc	*P*	Fc	*P*
AKR1B11	−3.511	−2.08	**	−2.50	*	−1.36	NS	−2.49	***
LAPTM4B	+3.058	+1.23	NS	+1.77	*	+1.31	*	−1.05	NS
EIF2AK3	−1.286	−1.64	*	−1.70	*	−1.18	NS	−1.34	NS
MIA2	−2.544	−1.54	*	−1.65	*	−1.14	NS	−1.35	*
EXOSC9	−1.503	+1.36	NS	−1.61	*	+1.14	NS	−1.24	NS
TJP1	−1.520	+1.66	NS	−1.66	*	+1.15	NS	−1.24	NS
XRN1	NS ($P < 0.08$)	+1.03	NS	−1.25	*	−1.02	NS	−1.14	*
SLC7A10	+1.263	−1.42	NS	+1.49	*	−1.76	NS	+2.52	*
SLC2A51	−1.259	−1.54	**	−1.05	NS	−1.72	**	−1.94	**
SLC16A1	NS	−1.11	NS	−1.10	NS	−1.37	*	−1.47	*
SLC27A4	+1.424	−1.43	NS	−1.18	NS	−1.68	NS	−1.87	*
IFT57	+1.953	+1.78	**	+1.33	NS	+1.38	NS	+1.41	NS
CASP1	+2.198	−1.13	NS	+1.04	NS	−1.43	**	−1.39	**
EP3001	+1.321	−1.26	NS	−1.60	NS	−1.52	***	−1.17	NS
MAPKAPK3	+1.299	−1.32	NS	−1.28	NS	−1.15	NS	−1.15	NS
STK39	+1.650	−1.03	NS	−1.53	NS	−1.22	NS	−1.01	NS
TP53I3	+2.646	−1.07	NS	+1.06	NS	+1.20	NS	−1.14	NS

RT = reverse transcription; DON = deoxynivalenol; Fc = fold change; *P* = *P*-value of Student's *t*-test.
n = 5 animals per treatment group.
Fc and *P* were measured between control and the corresponding DON-containing group.
[1]Raw value above 80th percentile.
$*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

the downregulation of *EIF2AK3* and *DNAJC3*. Pestka (2010) assumed that *EIF2AK3* might have an important role in the signalling after the challenge with DON, because of influenced up- and downstream signal transducers. The downregulation of *EIF2AK3* in our experiment confirms this assumption. The ribotoxic stress response seems to have some pathway elements similar to the ER stress response, but with inverse gene expression regulation.

Stabilisation of mRNA

The expression change of *EXOSC9* (fc: −1.503, DON5) was confirmed with qPCR (fc: −1.61) in the liver. EXOSC9 is a subunit of the exosome, a 3′–5′ exoribonuclease complex. This complex processes ribosomal and small nuclear RNAs and degrades mRNAs. In HeLa extracts, the addition of RNA containing an adenylate/uridylate-rich element (ARE) increased their 3′–5′ exonucleolytic decay rate. The inactivation of EXOSC9 highly decreased the efficiency of the exonucleolytic decay, showing the central role of EXOSC9 in mRNA decay (Mukherjee *et al.*, 2002). In human HEp-2 cells, it has been shown that the knockdown of EXOSC9 led to a twofold increase in *β-globulin* containing an ARE (van Dijk *et al.*, 2007). The 5′–3′ exoribonuclease *XRN1* (fc: −1.796, DON5) was numerically downregulated in the microarray experiment and significantly downregulated in qPCR experiment (fc: −1.25) in the liver. XRN1 is a component of the alternative 5′–3′ mRNA decay pathway (Schilders *et al.*, 2006). The knockdown of XRN1 led to a 3.5-fold increase of *the β-globulin-ARE* mRNA. Therefore, EXOSC9 and XRN1 seem to be involved in the turnover of ARE-containing transcripts (van Dijk *et al.*, 2007). In his review, Pestka (2008) summarised the genes with a lower mRNA decay rate upon DON administration, which are *COX-2*, tumour necrosis factor (*TNF*)-α and interleukin (*IL*)*-6* in macrophages and *IL-2* in EL-4T-cells. It has been reported that all four mRNAs are stabilised by ARE in the 3′ untranslated region (Chen *et al.*, 2001, Paschoud *et al.*, 2006, Rajasingh *et al.*, 2006). The downregulation of *EXOSC9* and *XRN1* might be the reason for increased mRNA stability of several ARE-containing genes in DON-challenged cell cultures. Further, the reduced mRNA depletion, due to *XRN1* and *EXOSC9* downregulation, might have also led to higher mRNA levels in general. In the final gene list (supplementary files), a disequilibrium exists with 367 upregulated genes and 199 downregulated genes. The possible higher mRNA amount might compensate for the possibly reduced translation, due to binding to the 60 S ribosomal subunit by DON. *EXOSC9* and *XRN1* seem to be interesting biomarkers for mRNA stabilisation and the decay rate of ARE-containing mRNA.

Tight junctions

Cells are connected by intercellular complexes such as tight junctions and adherens junctions. In Caco-2 cells, it has been shown that the transepithelial electrical resistance (TEER) decreased to 30% after 24 h of DON incubation at a concentration of 2 μg/ml. It was mentioned that TEER reflects the functional tight junctions made of transmembrane proteins

(Sergent *et al*., 2006). Among the proteins mentioned was TJP1 (fc: −1.52, DON5), which was downregulated in our experiment in the liver, which was also measured with qPCR (fc: −1.66). In porcine intestinal epithelial cells (IPEC-1) and -J2 cells, the treatment with 2 μg DON/ml led to disintegrated TJP1 structure and the total amount of TJP1 moderately decreased, which was measured with the Western blot analysis (Diesing *et al*., 2011). It was reported that TJP1 interacts with CLDN3 (fc: +1.637, DON1/5; Harhaj and Antonetti, 2004). Pinton *et al*. (2009) have shown a decreased CLDN3 protein amount in IPEC-1 after the treatment with DON for 48 h with a parallel decrease of TEER. Therefore, the upregulation of *CLDN3* might counterbalance the reduction caused by DON over a feedback mechanism. The decrease of TEER shows the acute effects in cell cultures. The extent to which the chronic administration of DON leads to the same effects has not been reported. The significant expression change of TJP1 and CLDN3 indicates that similar effects, such as the decreased TEER in cell cultures, might also occur in the liver of living animals.

Immune-related genes

The expression of *MIA2* (fc: −2.544, DON5) was significantly decreased, measured with qPCR in the liver (fc: −1.70) and the jejunum (fc: −1.35). *MIA2* expression is regulated by IL-6 and transforming growth factor-beta, which increased the expression level of *MIA2* in human hepatocytes and in HepG2 cells (Bosserhoff *et al*., 2003). The downregulation of *MIA2* in the liver and the jejunum may indicate a reduced amount of IL-6 in the liver cells of broilers. In murine macrophages, it has been shown that IL1RL1 (fc: +1.661, DON2.5/5) exerted anti-inflammatory effects by the negative regulation of the IL-6 production (Takezako *et al*., 2006), which affirmed the previous results with the decreased MIA2 expression. *In vitro* experiments with the addition of DON exhibited that the half-life of the ARE-containing IL-6 and TNF-α mRNA and the amount of the respective proteins were increased within 24 h (Wong *et al*., 2001). IL-6 seems to be critical for the regulation of immunoglobulin (Ig)A. In broilers fed with 7 to 9.2 mg DON/kg feed, the biliary IgA concentration was decreased (Swamy *et al*., 2002). Azcona-Olivera *et al*. (1995) showed an increased expression of *IL-1β*, *IL-6* and *TNF-α* mRNA in mice exposed to a 25 mg DON/kg diet. Four hours after the administration of DON, the cytokines were again only slightly upregulated in the liver (Azcona-Olivera *et al*., 1995). This may also explain the findings that *IL-1β*, *IL-6* and *TNF-α* mRNA were unchanged in our experiment. IL1RL1 might be an antagonist to DON by stabilising IL-6 and TNF-α levels. Especially in long-term administration of DON, the normalisation of the mRNA and cytokine levels might be economical. The upregulation of pro- and anti-inflammatory factors may influence the sensitivity of the immune system and act as immune stimulators. *LAPTM4B* (+3.058, DON1/5) expression increase could be confirmed in the liver (fc: +1.77, DON5) and the jejunum (fc: +1.31, DON2.5). LAPTM4B is a tetra-transmembrane glycoprotein localised on endosomes and lysosomes (Liu *et al*., 2009). LAPTM4B was also upregulated in the early phase of liver regeneration in rats (Xu *et al*., 2005). Therefore, LAPTM4B might be used as a biomarker for liver regeneration.

Conclusion

We could confirm several results in the living animal from previous studies, which were performed mainly in cell cultures. The main effects with economic impact might be the altered expression of the solute carrier transcripts, which transport D-glucose/D-fructose, palmitic acid, monocarboxylates, L-serine, leucine and other amino acids. Further, the translation initiation-related genes *EIF2AK3* and *DNAJC3*, as well as the mRNA stabilisation genes, could have an important influence on the protein synthesis rate and on the growth of broilers. The upregulation of DNA repair proteins indicates possible mutagenic effects of DON or its decomposition products. The detoxification enzyme AKR1B1 is an indicator that, at 2.21 mg DON/kg feed, the concentration might be high enough to show cytotoxic effects. The verification of the results in the jejunum from the microarray and the qPCR analysis in the liver increases the reliability of the DON-regulated genes. In general, effects on the level of RNA expression cannot be directly transposed from one organ to the other. Therefore, the parallel alterations in the liver and the jejunum might indicate a general functional mechanism of DON on the different relative amounts of transcripts. The maximum-allowed DON contamination in poultry feed in the European Union (2006) is 5 mg DON/kg feed. Significantly altered gene expression was observed at a concentration of 2.21 mg DON/kg feed.

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References

Abraham J, Lemmers B, Hande MP, Moynahan ME, Chahwan C, Ciccia A, Essers J, Hanada K, Chahwan R, Khaw AK, McPherson P, Shehabeldin A, Laister R, Arrowsmith C, Kanaar R, West SC, Jasin M and Hakem R 2003. Eme1 is involved in DNA damage processing and maintenance of genomic stability in mammalian cells. EMBO Journal 22, 6137–6147.

Aeschbacher K, Messikommer R, Meile L and Wenk C 2005. Bt176 corn in poultry nutrition: physiological characteristics and fate of recombinant plant DNA in chickens. Poultry Science 84, 385–394.

Awad WA, Bohm J, Razzazi-Fazeli E, Hulan HW and Zentek J 2004. Effects of deoxynivalenol on general performance and electrophysiological properties of intestinal mucosa of broiler chickens. Poultry Science 83, 1964–1972.

Awad WA, Ghareeb K, Bohm J, Razzazi E, Hellweg P and Zentek J 2008. The impact of the Fusarium toxin deoxynivalenol (DON) on poultry. International Journal of Poultry Science 7, 827–842.

Azcona-Olivera JI, Ouyang Y, Murtha J, Chu FS and Pestka JJ 1995. Induction of cytokine mRNAs in mice after oral exposure to the trichothecene vomitoxin

(deoxynivalenol): relationship to toxin distribution and protein synthesis inhibition. Toxicology and Applied Pharmacology 133, 109–120.

Bondy GS and Pestka JJ 2000. Immunomodulation by fungal toxins. Journal of Toxicology and Environmental Health, Part B: Critical Reviews 3, 109–143.

Bosserhoff AK, Moser M, Scholmerich J, Buettner R and Hellerbrand C 2003. Specific expression and regulation of the new melanoma inhibitory activity-related gene MIA2 in hepatocytes. Journal of Biological Chemistry 278, 15225–15231.

Chen CY, Gherzi R, Ong SE, Chan EL, Raijmakers R, Pruijn GJ, Stoecklin G, Moroni C, Mann M and Karin M 2001. AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. Cell 107, 451–464.

De Walle JV, Sergent T, Piront N, Toussaint O, Schneider YJ and Larondelle Y 2010. Deoxynivalenol affects in vitro intestinal epithelial cell barrier integrity through inhibition of protein synthesis. Toxicology and Applied Pharmacology 245, 291–298.

Diesing AK, Nossol C, Panther P, Walk N, Post A, Kluess J, Kreutzmann P, Danicke S, Rothkotter HJ and Kahlert S 2011. Mycotoxin deoxynivalenol (DON) mediates biphasic cellular response in intestinal porcine epithelial cell lines IPEC-1 and IPEC-J2. Toxicology Letters 200, 8–18.

EFSA 2004. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Deoxynivalenol as undesirable substance in animal feed. The EFSA Journal 89, 1–35.

European Union 2006. 2006/576/EC: Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. Official Journal – European Union Legislation 49, 7–9.

Frankic T, Pajk T, Rezar V, Levart A and Salobir J 2006. The role of dietary nucleotides in reduction of DNA damage induced by T-2 toxin and deoxynivalenol in chicken leukocytes. Food and Chemical Toxicology 44, 1838–1844.

Gale M Jr, Blakely CM, Hopkins DA, Melville MW, Wambach M, Romano PR and Katze MG 1998. Regulation of interferon-induced protein kinase PKR: modulation of P58IPK inhibitory function by a novel protein, P52rIPK. Molecular and Cellular Biology 18, 859–871.

Harhaj NS and Antonetti DA 2004. Regulation of tight junctions and loss of barrier function in pathophysiology. International Journal of Biochemistry & Cell Biology 36, 1206–1237.

Jin Y and Penning TM 2007. Aldo-keto reductases and bioactivation/detoxication. Annual Review of Pharmacology and Toxicology 47, 263–292.

Kanai Y, Segawa H, Miyamoto K, Uchino H, Takeda E and Endou H 1998. Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). Journal of Biological Chemistry 273, 23629–23632.

Kawata K, Yokoo H, Shimazaki R and Okabe S 2007. Classification of heavy-metal toxicity by human DNA microarray analysis. Environmental Science and Technology 41, 3769–3774.

Lambert DW, Wood IS, Ellis A and Shirazi-Beechey SP 2002. Molecular changes in the expression of human colonic nutrient transporters during the transition from normality to malignancy. British Journal of Cancer 86, 1262–1269.

Liu X, Xiong F, Wei X, Yang H and Zhou R 2009. LAPTM4B-35, a novel tetratransmembrane protein and its PPRP motif play critical roles in proliferation and metastatic potential of hepatocellular carcinoma cells. Cancer 100, 2335–2340.

Lun AK, Moran ET Jr, Young LG and McMillan EG 1989. Absorption and elimination of an oral dose of 3H-deoxynivalenol in colostomized and intact chickens. Bulletin of Environmental Contamination and Toxicology 42, 919–925.

Maresca M, Mahfoud R, Garmy N and Fantini J 2002. The mycotoxin deoxynivalenol affects nutrient absorption in human intestinal epithelial cells. Journal of Nutrition 132, 2723–2731.

Marzocco S, Russo R, Bianco G, Autore G and Severino L 2009. Pro-apoptotic effects of nivalenol and deoxynivalenol trichothecenes in J774A.1 murine macrophages. Toxicology Letters 189, 21–26.

Mello JA, Sillje HH, Roche DM, Kirschner DB, Nigg EA and Almouzni G 2002. Human Asf1 and CAF-1 interact and synergize in a repair-coupled nucleosome assembly pathway. EMBO Reports 3, 329–334.

Miller KA, Yoshikawa DM, McConnell IR, Clark R, Schild D and Albala JS 2002. RAD51C interacts with RAD51B and is central to a larger protein complex in vivo exclusive of RAD51. Journal of Biological Chemistry 277, 8406–8411.

Mukherjee D, Gao M, O'Connor JP, Raijmakers R, Pruijn G, Lutz CS and Wilusz J 2002. The mammalian exosome mediates the efficient degradation of mRNAs that contain AU-rich elements. EMBO Journal 21, 165–174.

Nakauchi J, Matsuo H, Kim DK, Goto A, Chairoungdua A, Cha SH, Inatomi J, Shiokawa Y, Yamaguchi K, Saito I, Endou H and Kanai Y 2000. Cloning and characterization of a human brain Na(+)-independent transporter for small neutral amino acids that transports D-serine with high affinity. Neuroscience Letters 287, 231–235.

National Research Council 1994. Nutrient requirements of poultry. National Academy Press, Washington, DC.

O'Brien PJ and Ellenberger T 2004. Dissecting the broad substrate specificity of human 3-methyladenine-DNA glycosylase. Journal of Biological Chemistry 279, 9750–9757.

Paschoud S, Dogar AM, Kuntz C, Grisoni-Neupert B, Richman L and Kuhn LC 2006. Destabilization of interleukin-6 mRNA requires a putative RNA stem-loop structure, an AU-rich element, and the RNA-binding protein AUF1. Molecular and Cellular Biology 26, 8228–8241.

Pepper SD, Saunders EK, Edwards LE, Wilson CL and Miller CJ 2007. The utility of MAS5 expression summary and detection call algorithms. BMC Bioinformatics 8, 273.

Pestka JJ 2007. Deoxynivalenol: toxicity, mechanisms and animal health risks. Animal Feed Science and Technology 137, 283–298.

Pestka JJ 2008. Mechanisms of deoxynivalenol-induced gene expression and apoptosis. Food Additives and Contaminants, 1–13.

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

Pinton P, Nougayrede JP, Del Rio JC, Moreno C, Marin DE, Ferrier L, Bracarense AP, Kolf-Clauw M and Oswald IP 2009. The food contaminant deoxynivalenol, decreases intestinal barrier permeability and reduces claudin expression. Toxicology and Applied Pharmacology 237, 41–48.

Plummer ER and Calvert H 2007. Targeting poly(ADP-ribose) polymerase: a two-armed strategy for cancer therapy. Clinical Cancer Research 13, 6252–6256.

Prelusky DB, Gerdes RG, Underhill KL, Rotter BA, Jui PY and Trenholm HL 1994. Effects of low-level dietary deoxynivalenol on haematological and clinical parameters of the pig. Natural Toxins 2, 97–104.

Rajasingh J, Bord E, Luedemann C, Asai J, Hamada H, Thorne T, Qin G, Goukassian D, Zhu Y, Losordo DW and Kishore R 2006. IL-10-induced TNF-alpha mRNA destabilization is mediated via IL-10 suppression of p38 MAP kinase activation and inhibition of HuR expression. FASEB Journal 20, 2112–2114.

Robbana-Barnat S, Loridon-Rosa B, Cohen H, Lafarge-Frayssinet C, Neish GA and Frayssinet C 1987. Protein synthesis inhibition and cardiac lesions associated with deoxynivalenol ingestion in mice. Food Additives and Contaminants 4, 49–56.

Ron D 2002. Translational control in the endoplasmic reticulum stress response. Journal of Clinical Investigation 110, 1383–1388.

Rotter BA, Prelusky DB and Pestka JJ 1996. Toxicology of deoxynivalenol (vomitoxin). Journal of Toxicology and Environmental Health 48, 1–34.

Schilders G, van Dijk E, Raijmakers R and Pruijn GJ 2006. Cell and molecular biology of the exosome: how to make or break an RNA. International Review of Cytology 251, 159–208.

Scott TA and Hall JW 1998. Using acid insoluble ash marker ratios (diet : digesta) to predict digestibility of wheat and barley metabolizable energy and nitrogen retention in broiler chicks. Poultry Science 77, 674–679.

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

Shifrin VI and 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. Journal of Biological Chemistry 274, 13985–13992.

Shimamoto T, Tanimura T, Yoneda Y, Kobayakawa Y, Sugasawa K, Hanaoka F, Oka M, Okada Y, Tanaka K and Kohno K 1995. Expression and functional analyses of the Dxpa gene, the Drosophila homolog of the human excision repair gene XPA. Journal of Biological Chemistry 270, 22452–22459.

Silverman J, Takai H, Buonomo SB, Eisenhaber F and de Lange T 2004. Human Rif1, ortholog of a yeast telomeric protein, is regulated by ATM and 53BP1 and functions in the S-phase checkpoint. Genes and Development 18, 2108–2119.

Stahl A, Hirsch DJ, Gimeno RE, Punreddy S, Ge P, Watson N, Patel S, Kotler M, Raimondi A, Tartaglia LA and Lodish HF 1999. Identification of the major intestinal fatty acid transport protein. Molecular Cell 4, 299–308.

Swamy HV, Smith TK, Karrow NA and Boermans HJ 2004. Effects of feeding blends of grains naturally contaminated with Fusarium mycotoxins on growth and immunological parameters of broiler chickens. Poultry Science 83, 533–543.

Swamy HV, Smith TK, Cotter PF, Boermans HJ and Sefton AE 2002. Effects of feeding blends of grains naturally contaminated with Fusarium mycotoxins on production and metabolism in broilers. Poultry Science 81, 966–975.

Takezako N, Hayakawa M, Hayakawa H, Aoki S, Yanagisawa K, Endo H and Tominaga S 2006. ST2 suppresses IL-6 production via the inhibition of IkappaB degradation induced by the LPS signal in THP-1 cells. Biochemical and Biophysical Research Communications 341, 425–432.

Ueno Y 1984. Toxicological features of T-2 toxin and related trichothecenes. Fundamental and Applied Toxicology 4, S124–S132.

van Dijk EL, Schilders G and Pruijn GJ 2007. Human cell growth requires a functional cytoplasmic exosome, which is involved in various mRNA decay pathways. RNA 13, 1027–1035.

van Huizen R, Martindale JL, Gorospe M and Holbrook NJ 2003. P58IPK, a novel endoplasmic reticulum stress-inducible protein and potential negative regulator of eIF2alpha signaling. Journal of Biological Chemistry 278, 15558–15564.

Wong S, Schwartz RC and Pestka JJ 2001. Superinduction of TNF-alpha and IL-6 in macrophages by vomitoxin (deoxynivalenol) modulated by mRNA stabilization. Toxicology 161, 139–149.

Xu CS, Chang CF, Yuan JY, Li WQ, Han HP, Yang KJ, Zhao LF, Li YC, Zhang HY, Rahman S and Zhang JB 2005. Expressed genes in regenerating rat liver after partial hepatectomy. World Journal of Gastroenterology 11, 2932–2940.

Yang GH, Li S and Pestka JJ 2000. Down-regulation of the endoplasmic reticulum chaperone GRP78/BiP by vomitoxin (Deoxynivalenol). Toxicology and Applied Pharmacology 162, 207–217.

Yoshizawa T, Cote LM, Swanson SP and and Buck WB 1986. Confirmation of DOM-1, a de-epoxidation metabolite of deoxynivalenol, in biological fluids of lactating cows. Agricultural and Biological Chemistry 50, 227–229.