Dietary deoxynivalenol and oral lipopolysaccharide challenge differently affect intestinal innate immune response and barrier function in broiler chickens¹

Annegret Lucke, Josef Böhm, Qendrim Zebeli, and Barbara U. Metzler-Zebeli²

Department of Farm Animals and Veterinary Public Health, Institute of Animal Nutrition and Functional Plant Compounds, University of Veterinary Medicine Vienna, 1210 Vienna, Austria

ABSTRACT: Dietary deoxynivalenol (DON) impairs the intestinal immune system and digestive functions of broiler chickens. However, little is known whether increasing doses of DON similarly affect the intestinal functions in different segments of the small intestine in chickens and whether a second oral challenge may potentiate those effects. The present objective was to investigate the effect of increasing dietary DON concentrations on the relative expression of genes for tight junction proteins, mucins, toll-like receptors (TLR), and cytokines in duodenum and jejunum, jejunal mucosal permeability, as well as on α -1-acid glycoprotein and IgA in serum with or without an additional oral lipopolysaccharide (LPS) challenge. Eighty 1-d-old chickens were fed diets with increasing DON concentrations (0, 2.5, 5, and 10 mg DON/kg diet) for 5 wk. One day before sampling, half of the chickens received an oral challenge with 1-mg *Escherichia coli* O55:B5 LPS/kg BW. Ussing chambers were used to measure the jejunal permeability in birds receiving 10-mg DON/kg feed with or without LPS challenge and

0-mg DON/kg feed without LPS. Increasing DON concentrations of up to 5-mg DON/kg increased ($P < 0.05$) the duodenal expression of *TLR2*, *IL6*, and Claudin 1 (*CLDN1*) by up to 84%, 88%, and 48%, respectively, compared with the noncontaminated diet. Likewise, jejunal *CLDN1* expression increased up to 23% in the chickens fed DON concentrations of up to 5-mg DON/kg diet ($P < 0.05$). Moreover, increasing DON concentrations linearly and quadratically decreased ($P < 0.05$) the jejunal expression of *TLR2* and transforming growth factor- β 1, respectively. The additional LPS challenge increased ($P < 0.040$) duodenal occludin expression by 10% as well as the jejunal tissue conductance in chickens of the 10 DON group ($P = 0.050$). In conclusion, dietary DON differently affected the duodenal and jejunal expression of genes for tight-junction proteins and proinflammatory signaling pathways. The additional LPS challenge did not potentiate the DON effect but it seemed to induce a certain up-regulation of the proinflammatory response in the duodenum and enhanced the mucosal permeability in the jejunum.

Key words: broiler chicken, deoxynivalenol, intestinal barrier, innate immune response, lipopolysaccharide, Ussing chamber

© The Author(s) 2018. Published by Oxford University Press on behalf of the American Society of Animal Science. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.

J. Anim. Sci. 2018.96:5134–5143

doi: 10.1093/jas/sky379

¹This project has received funding from the Austrian Research Promotion Agency (FFG; project number 848446) and Biomin GmbH. We would like to thank Arife Sener, Suchitra Sharma, Manfred Hollmann, Melanie Wild, Anita Dockner, Barbara Doupovec, and Georg Kvapil for assistance in laboratory analyses, diet formulation, and the

animal trial. We declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

²Corresponding author: Barbara.Metzler@vetmeduni.ac.at

Received June 8, 2018.

Accepted October 4, 2018.

INTRODUCTION

Deoxynivalenol (DON) is a ubiquitous mycotoxin with negative effects on the growth performance of broiler chickens (Andretta et al., 2011; EFSA, 2013). Being an inhibitor of the protein, RNA, and DNA synthesis, DON mainly affects cells with a high-protein turnover, such as intestinal epithelial and immune cells (Awad et al., 2013). Although DON-related effects on the mucosal barrier function in the upper small intestine have been previously described (Osselaere et al., 2013; Ghareeb et al., 2015), less is known about the dose- and intestinal site-dependent effects of DON on the toll-like receptor (TLR) response and cytokine expression in chickens. Concurrently, chickens are exposed to lipopolysaccharides (LPS), another ubiquitous immune stimulant originating from the environment, feed, and intestinal microbiota (Ganey and Roth, 2001; Wallace et al., 2016). In chickens, oral LPS exposure induces intestinal cytokine expression (Wu et al., 2013) and interfere in response to other xenobiotic agents, such as DON (Xu et al. 2011). However, possible interactions between DON and oral LPS on the innate immunity in broiler chickens have not been elucidated so far. We hypothesized that, due to the progressing detoxification of DON in the upper digestive tract (Awad et al., 2011), chronic exposure to increasing levels of DON in chicken feed would compromise the intestinal barrier function and alter the innate immune response in the duodenum more severely than in the jejunum (Antonissen et al., 2014, 2015). We further assumed that an additional immune challenge with LPS would aggravate the DON effects on the intestinal epithelium. Our objective was to investigate the impact of increasing DON concentrations on the relative expression of genes for tight junction proteins, mucins, TLRs, and cytokines in duodenum and jejunum, jejunal mucosal permeability, and the serum acute-phase-protein α -1-acid glycoprotein (AGP) and IgA with or without an additional LPS challenge.

MATERIALS AND METHODS

Ethics Statement

The animal procedures were approved by the institutional ethics committee of the University of Veterinary Medicine Vienna and the Austrian National Authority according to paragraph 26 of Law for Animal Experiments, Tierversuchsgesetz 2012–TVG 2012 (GZ 68.205/0062–WF/V/3b/2015).

Experimental Design

The feeding experiment, housing conditions, diets, and performance parameters are described in Lucke et al. (2017). In brief, eighty 1-d-old broiler chicks (ROSS 308) obtained from a commercial hatchery (Brütereier Schlierbach GmbH, Pettenbach, Austria) were used in a completely randomized 4×2 factorial design with 3 replicate batches. Chickens were randomly assigned to 1 out of 4 feeding groups between the first day of life and 5 wk of age: 1) control diet without DON (0 DON), 2) control diet experimentally contaminated with 2.5-mg DON (Romer Labs, Tulln, Austria) per kg feed (2.5 DON), 3) control diet with 5-mg DON/kg feed (5 DON), and 4) control diet with 10-mg DON/kg feed (10 DON). Diets were fed ad libitum from day 1 of life until the end of the experimental period (days 34 to 37 of life). One day before sample collection, half of the birds within each feeding group received an oral LPS treatment at a dose of 1-mg LPS/kg BW (LPS L2880 from *Escherichia coli* O55:B5, Sigma-Aldrich, St Louis, MO) via crop gavage. Non-LPS-treated birds received distilled water as placebo. This resulted in the following 8 treatment groups (4×2 factorial arrangement): 0 DON + con, 0 DON + lps, 2.5 DON + con, 2.5 DON + lps, 5 DON + con, 5 DON + lps, 10 DON + con, and 10 DON + lps.

Chickens were housed in flatdeck cages (0.36 m² each) in groups of 2 to 4 animals per cage from weeks 1 to 3 of the experiment and 1 to 2 birds per cage for the remaining time of the experiment (Lucke et al., 2017, 2018). Cardboard was used as bedding material. The light program consisted of 23 h of light in the first 6 d, followed by 2 d with a 22-h light period and 20 h of light for the rest of the trial. Temperature management was according to the Ross Aviagen Management Handbook (Aviagen, 2014), starting at 33 °C on day 1 of life and gradually decreasing to 20 °C on day 27 of life.

Sample Collection

Feed was withdrawn for 0.5 to 1.5 h before chickens were euthanized between days 34 and 37 of life by an overdose of Thiopental (50 to 100 mg/kg BW; Thiopental medicamentum pharma GmbH, Allerheiligen im Mürtztal, Austria) into the wing vein and subsequent exsanguination. Blood was collected in serum tubes (4 mL, Sarstedt AG + Co., Nümbrecht, Germany) during exsanguination and allowed to clot before centrifugation at $2,000 \times g$ for 10 min. Immediately after the death of the animal,

the entire intestine was carefully removed and the respective segments from the small intestine for the gene expression and Ussing chamber experiments were collected. For the gene expression experiment, the duodenum (from pylorus to the end of the pancreatic loop) and proximal part of the jejunum (30 cm taken from the beginning of the jejunum) were excised, opened longitudinally at the mesenteric border, immediately washed in sterile, ice-cold phosphate-buffered saline, and carefully blotted dry with paper towel. The mucosa was scraped with a sterile glass microscopic slide and was immediately snap-frozen in liquid nitrogen within 20 min after death of the animal and stored at -80°C until analysis. For the Ussing chamber experiment, a 20-cm tube piece was taken distal from the Meckel's diverticulum, immediately transferred into ice-cold transport buffer (Supplementary Table S2) and gassed with carbogen gas (95% O_2 and 5% CO_2) for transport to the Ussing chamber laboratory, within 10 min after death.

RNA Isolation, Complementary DNA Synthesis, and Quantitative PCR

The RNA isolation, complementary DNA (cDNA) synthesis, and quantitative PCR (qPCR) were performed as recently described (Newman et al., 2018). Total RNA was isolated from snap-frozen mucosa scrapings (20 mg) of the duodenum and jejunum using the RNeasy Mini QIAcube kit on the QIAcube robotic work station (Qiagen, Hilden, Germany) with an additional homogenization step using the FastPrep-24 instrument (MP Biomedicals, Santa Ana, CA). Genomic DNA was removed with the Turbo DNA kit (Life Technologies Limited, Vienna, Austria), RNA was quantified using the Qubit HS RNA Assay kit on the Qubit 2.0 Fluorometer (Life Technologies), and the RNA quality was measured with the Agilent Bioanalyzer 2100 (Agilent RNA 6000 Nano Assay, Agilent Technologies, Waghäusel-Wiesental, Germany). The majority of duodenal and jejunal samples had RNA integrity numbers (RIN) between 8.0 and 10.0; nine samples had RINs between 7.0 and 7.9. The cDNA was synthesized from 2- μg RNA using the High Capacity cDNA RT kit (Life Technologies Limited, Vienna, Austria) with 1 μL of RNase inhibitor (Biozym, Hessisch Oldendorf, Germany) added to each reaction. The amplifications were performed in duplicates on the C1000 Touch Thermal Cycler (Bio-Rad, Vienna, Austria). Each reaction contained 50-ng cDNA, 10- μL Fast Plus Eva Green master mix with low ROX (Biotium, Hayward,

CA), 10- μL DEPC-treated water, and 100 nM each of forward and reverse primers (Supplementary Table S1).

The 16 target genes selected were related to the intestinal innate immune response and comprised genes for TLR-signaling, pro- and anti-inflammatory cytokines, apoptosis, and barrier function. Primers were newly designed and, similar to the previously published primers, verified using Primer BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast/, accessed January 02, 2018). In addition, they were tested for efficiencies and specificity using dissociation curve analysis. Negative template controls and RT minus controls using RNA were run on each plate. To determine the qPCR efficiencies $[(10^{(-1/\text{slope})} - 1) \times 100]$, 5-fold serial dilutions of pooled cDNA samples were performed. From the 5 housekeeping genes [HKG; beta-actin (*ACTB*), beta-2-microglobulin (*B2M*), hypoxanthine phosphoribosyltransferase 1 (*HPRT1*), glyceraldehydes-3-phosphate dehydrogenase (*GAPDH*), and small nuclear ribonucleoprotein D3 polypeptide (*SNRPD3*)], the 2 most stably expressed HKG (*B2M*, *GAPDH*) were determined using NormFinder (Andersen et al., 2004) and BestKeeper (Pfaffl et al., 2004). The geometric mean expression level of those 2 HKG was used to calculate the target gene expression according to the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001). To obtain the ΔC_t value, the geometric mean of the C_t values of the HKG was subtracted from the C_t value of the target gene. Afterwards, ΔC_t value of the chicken with the lowest ΔC_t value was used for calculation of the $\Delta\Delta C_t$ value.

Ussing Chamber Experiment

The electrophysiological parameters were evaluated for the distal jejunum of chickens from the 0 DON + con, 10 DON + con, and 10 DON + lps groups. A detailed description of the intestinal electrophysiological measurements can be found in Metzler-Zebeli et al. (2017). Briefly, mucosal preparations were used in Ussing chambers (diam. 0.91 cm^2 ; buffer compositions are given in Supplementary Table S2), with 4 successional samples from the jejunal tube piece being evaluated in parallel. Electrophysiological parameters were recorded with a computer-aided data-recording package (Mussler Scientific Instruments, Aachen, Germany). After an equilibration period of 20 min under open-circuit conditions, the chambers were voltage-clamped to 0 mV and electrophysiological measurements of short-circuit current (I_{sc} ,

$\mu\text{A}/\text{cm}^2$), transepithelial resistance ($\Omega \times \text{cm}^2$), and potential difference (mV) were continuously recorded. Tissue conductance (G_T , mS/cm^2) was calculated as the reciprocal value of the tissue resistance. At 185 min, 14.4 mg of theophylline (final concentration 8 mmol/L in chamber half; Sigma-Aldrich, St Louis, MO) was added to both serosal and mucosal sides of the Ussing chamber, to verify the viability of the tissue at the end of the experiment. Tissue preparations that did not show an electrophysiological response after theophylline were dismissed (3.3%).

Determination of Serum α -1-Acid Glycoprotein and IgA

Chicken-specific commercially available ELISA assay kits were used to measure AGP (GenWay Biotech, Inc., San Diego, CA) and IgA (Bethyl Laboratories, Inc., Montgomery, TX) in serum according to the manufacturers' instructions. The samples were diluted 1:12,500 and 1:1,000 for the AGP and IgA assays, respectively, and the absorbance was measured with an iMark absorbance reader (Bio-Rad Laboratories, Munich, Germany). All samples were measured in duplicate. The coefficient of variation between duplicate measurements was less than 6%.

Statistical Analysis

Normal distribution of the residuals was checked by the Shapiro–Wilk test using the UNIVARIATE procedure in SAS (Version 9.4; SAS Institute, Inc., Cary, NC). Statistical analyses of the relative gene expression, mucosal barrier function, and serum parameters were performed using the MIXED procedure of SAS (Version 9.4; SAS Institute, Inc., Cary, NC). For Ussing chamber data, the model accounted for treatment as a fixed effect and replicate batch as a random effect. The experimental unit was defined as chicken nested within group and day with the covariance structure being variance compounds. For gene expression, serum IgA and AGP data, DON treatment, LPS, and their 2-way-interaction were considered as fixed effects. The replicate batch was included as random effect and chicken nested within treatment and day as the experimental unit. Linear and quadratic relationships between control feeding and the 3 increasing levels of DON-feeding and the overall difference of 0 DON vs. all DON groups were tested by calculating orthogonal contrasts using the CONTRAST statement of SAS. Degrees of

freedom were approximated using Kenward–Rogers method (ddfm = kr) for all parameters. Results are presented as least squares means $\pm$ SEM. Pairwise comparisons among least squares means were performed using the probability of difference (pdiff) option in SAS. Significance was declared if $P < 0.05$ and a trend was reported if $P < 0.10$.

RESULTS

The performance of the present chickens can be found in Lucke et al. (2017). Briefly, chickens fed the 5- and 10-mg DON/kg diet had a decreased DMI by 13% in the last experimental week compared with chickens fed the 0 DON diet ($P < 0.050$). The increasing DON concentrations further tended ($P = 0.082$) to decrease the final BW of chickens (1812, 1708, 1691, and 1712 $\pm$ 53 g in the 0, 2.5, 5, and 10 DON groups, respectively).

Expression of Target Genes in the Duodenal and Jejunal Mucosa

The increasing dietary DON concentrations affected ($P < 0.050$) the expression of *CLDN1*, *TLR2*, and *IL6* in the duodenal mucosa and of *CLDN1*, *TLR2*, and *TGFB1* in the jejunal mucosa (Tables 1 and 2). More specifically, increasing DON concentrations ($P \leq 0.010$) up-regulated the duodenal and jejunal expression of *CLDN1* by 48% and 23%, respectively (Table 1). Moreover, increasing dietary DON concentrations tended ($P < 0.10$) to increase duodenal *TJPI* and *MUC1* expression by up to 25% and 97%, respectively. Similarly, increasing DON concentrations up-regulated ($P < 0.050$) the expression levels of *TLR2* in the duodenum by up to 84% with a dietary DON concentration of 5 mg/kg, whereas it linearly down-regulated its expression in the jejunum by 49% (Table 2). The DON-fed birds further tended to have a 42% greater duodenal *TLR4* expression compared with the birds in the 0 DON group ($P = 0.084$). Also, the dietary DON up-regulated ($P = 0.001$) the expression of *IL6* by 88% and tended ($P = 0.082$) to linearly increase the *NFKB* expression by 26% in the duodenum (Table 2). By contrast, increasing DON concentrations quadratically affected the jejunal expression levels of *TGFB1* ($P = 0.038$) and, as trends, of *IL8* and *IL10* ($P < 0.010$) with the greatest down-regulation of the expression observed for the 2.5 DON group. The oral LPS challenge increased ($P = 0.040$) the duodenal *OCN* expression by 9.7% and tended ($P < 0.010$) to increase the duodenal *TNF* expression by 9.3%, whereas LPS

Table 1. Least squares means of relative expression of genes related to barrier function in duodenum and jejunum of broiler chickens fed diets with increasing levels of deoxynivalenol (DON; 0, 2.5, 5, or 10 mg DON/kg diet) and with or without oral lipopolysaccharide (LPS) challenge 1 day before sampling

Item ¹	no LPS				LPS				Pooled SEM	Fixed effect, <i>P</i> values			Contrasts, <i>P</i> values ²		
	DON, mg/kg feed									DON	LPS	DON × LPS	0 vs. DON	lin.	quad.
	0	2.5	5	10	0	2.5	5	10							
Duodenum															
<i>CLDN1</i>	0.384	0.454	0.547	0.459	0.381	0.484	0.585	0.525	0.052	0.010	0.380	0.937	0.005	0.013	0.034
<i>CLDN5</i>	0.179	0.238	0.303	0.172	0.169	0.212	0.270	0.241	0.058	0.276	0.996	0.800	0.177	0.390	0.115
<i>OCLN</i>	0.650	0.579	0.563	0.644	0.627	0.644	0.715	0.685	0.040	0.610	0.040	0.186	0.994	0.404	0.346
<i>TJPI</i>	0.430	0.489	0.545	0.493	0.453	0.507	0.557	0.499	0.046	0.141	0.651	0.998	<i>0.057</i>	0.143	<i>0.090</i>
<i>MUC1</i>	0.068	0.072	0.140	0.080	0.063	0.059	0.084	0.178	0.041	0.299	0.838	0.290	0.286	<i>0.074</i>	0.771
<i>MUC2</i>	0.219	0.190	0.304	0.252	0.257	0.201	0.263	0.236	0.044	0.264	0.957	0.834	0.932	0.450	0.963
Jejunum															
<i>CLDN1</i>	0.467	0.506	0.560	0.542	0.484	0.494	0.614	0.570	0.039	0.024	0.438	0.873	0.028	0.010	0.327
<i>CLDN5</i>	0.396	0.341	0.249	0.424	0.321	0.279	0.278	0.217	0.080	0.697	0.168	0.532	0.355	0.527	0.352
<i>OCLN</i>	0.590	0.545	0.532	0.593	0.543	0.602	0.636	0.662	0.044	0.513	0.146	0.353	0.433	0.169	0.558
<i>TJPI</i>	0.439	0.373	0.368	0.459	0.488	0.434	0.509	0.352	0.050	0.581	0.309	0.104	0.250	0.385	0.710
<i>MUC1</i>	3.033	2.168	2.102	3.484	1.992	1.155	2.069	1.537	0.817	0.686	<i>0.085</i>	0.712	0.524	0.872	0.273
<i>MUC2</i>	0.397	0.388	0.410	0.360	0.411	0.247	0.307	0.298	0.066	0.560	0.120	0.675	0.203	0.378	0.546

Values are least square means and pooled SEM; *n* = 9 in the 0 DON + con group for parameters in the duodenum, and *n* = 10 for all remaining groups. Significant *P* values (*P* < 0.05) are bold, *P* < 0.10 are italicized.

¹*CLDN1*, claudin 1; *CLDN5*, claudin 5; *OCN*, occluding; *TJPI*, tight junction protein 1; *MUC1*, mucin 1; *MUC2*, mucin 2.

²*P* values for orthogonal contrasts to test linear (lin.) and quadratic (quad.) relationships between control feeding and the three increasing levels of DON as well as the overall difference between 0 DON and all DON groups (0 vs. DON).

decreased the jejunal *MUC1* by 37% compared with placebo-treated birds, respectively (Table 1).

Ussing Chamber Experiment

Electrogenic ion transport and tissue permeability were determined in the 0 DON + con, 10 DON + con and 10 DON + lps groups using I_{sc} and G_T (Table 3). The 10 DON + lps treatment increased (*P* < 0.050) the G_T in chickens by 72% compared with 10 DON alone, but did not alter the I_{sc} .

Serum Concentrations of α -1-Acid Glycoprotein and IgA

Dietary DON and the LPS treatment did not affect serum AGP (Table 4), whereas the serum IgA concentration tended (*P* = 0.075) to linearly increase with increasing DON-concentrations by up to 41%.

DISCUSSION

There is growing evidence from in vitro studies and mammals (Pestka et al., 2007) that DON enhances the transcription of proinflammatory cytokines and activation via the phosphorylation of mitogen-activated protein kinases (MAPK) in a dose-dependent manner. Although the impact of DON on the mucosal barrier function in the

small intestine and digestive functions have been described in chickens, dose–response relationships of DON on the chicken innate immune response related to TLR2 and TLR4 signaling have been not sufficiently elucidated in chickens. Present results demonstrate that DON differently affected the expression of tight-junction protein genes and genes within proinflammatory signaling pathways in the duodenum and jejunum of chickens when exposed to graded levels of DON from early in life. However, for some of the genes that were differently expressed with DON, we did not find dose–response relationships but quadratic effects towards the dietary DON (e.g., *TGFB1*, *IL8*, and *IL10*). This may have been partly associated with the decrease in DMI of chickens exposed to the highest DON concentrations (Lucke et al., 2017). However, serum IgA levels were greatest in chickens being exposed to the highest DON concentration (10 mg/kg), which may have indicated an increased intestinal absorption of and immune stimulating effect of DON compared with the other DON concentrations. Several DON-associated effects have been reported to decrease the DMI in chickens when exposed to DON including an altered immune response (Chen et al., 2017), impaired digestive functions (Ghareeb et al., 2015), changes in the intestinal microbiota (Lucke et al., 2018),

Table 2. Least squares means of relative expression of genes related to toll-like receptor and cytokine signaling in duodenum and jejunum of broiler chickens fed diets with increasing levels of deoxynivalenol (DON; 0, 2.5, 5, or 10 mg DON/kg diet) and with or without oral lipopolysaccharide (LPS) challenge 1 d before sampling

Item ¹	no LPS				LPS				pooled SEM	Fixed effect, <i>P</i> values			Contrasts, <i>P</i> values ²		
	DON, mg/kg feed									DON	LPS	DON × LPS	0 vs. DON	lin.	quad.
	0	2.5	5	10	0	2.5	5	10							
Duodenum															
<i>TLR2</i>	0.158	0.197	0.297	0.157	0.121	0.190	0.217	0.272	0.049	0.125	0.952	0.224	0.046	<i>0.067</i>	0.166
<i>TLR4</i>	0.285	0.331	0.405	0.329	0.295	0.396	0.421	0.393	0.061	0.271	0.378	0.950	<i>0.084</i>	0.185	0.153
<i>NFKB</i>	0.291	0.234	0.365	0.334	0.291	0.311	0.334	0.400	0.054	0.250	0.468	0.712	0.393	<i>0.082</i>	0.644
<i>IL1B</i>	0.081	0.092	0.186	0.089	0.103	0.159	0.142	0.186	0.049	0.533	0.307	0.498	0.215	0.262	0.387
<i>IL6</i>	0.302	0.234	0.475	0.380	0.230	0.304	0.526	0.394	0.062	0.001	0.719	0.676	0.022	0.003	0.187
<i>IL8</i>	0.076	0.053	0.110	0.083	0.129	0.147	0.068	0.105	0.048	0.993	0.358	0.544	0.842	0.819	0.909
<i>IL10</i>	0.085	0.103	0.036	0.107	0.120	0.039	0.146	0.077	0.047	0.930	0.702	0.270	0.655	0.939	0.636
<i>TNF</i>	0.609	0.538	0.530	0.568	0.584	0.595	0.598	0.678	0.040	0.406	<i>0.068</i>	0.411	0.725	0.541	0.121
<i>TGFB1</i>	0.247	0.280	0.388	0.230	0.240	0.244	0.259	0.313	0.051	0.443	0.536	0.222	0.318	0.370	0.331
<i>CAS</i>	0.530	0.532	0.534	0.591	0.576	0.532	0.568	0.471	0.050	0.952	0.782	0.334	0.716	0.764	0.992
Jejunum															
<i>TLR2</i>	0.341	0.270	0.202	0.194	0.268	0.162	0.313	0.119	0.060	<i>0.096</i>	0.390	0.258	<i>0.058</i>	0.037	0.881
<i>TLR4</i>	0.462	0.456	0.426	0.488	0.410	0.407	0.406	0.393	0.070	0.987	0.278	0.960	0.912	0.993	0.777
<i>NFKB</i>	0.425	0.364	0.425	0.451	0.398	0.363	0.360	0.328	0.057	0.865	0.180	0.720	0.522	0.835	0.577
<i>IL1B</i>	0.342	0.232	0.192	0.337	0.236	0.206	0.243	0.214	0.064	0.568	0.265	0.515	0.329	0.840	0.163
<i>IL6</i>	0.399	0.350	0.490	0.533	0.471	0.411	0.384	0.378	0.059	0.622	0.447	0.137	0.821	0.536	0.385
<i>IL8</i>	0.284	0.142	0.208	0.332	0.203	0.128	0.170	0.171	0.063	0.232	0.104	0.670	0.320	0.700	<i>0.060</i>
<i>IL10</i>	0.137	0.032	0.028	0.099	0.072	0.007	0.017	0.032	0.047	0.240	0.217	0.913	<i>0.080</i>	0.447	<i>0.062</i>
<i>TNF</i>	0.650	0.563	0.566	0.554	0.561	0.560	0.540	0.590	0.041	0.587	0.477	0.475	0.194	0.401	0.272
<i>TGFB1</i>	0.352	0.228	0.227	0.320	0.236	0.188	0.245	0.250	0.045	0.188	0.107	0.506	0.169	0.998	0.038
<i>CAS</i>	0.242	0.282	0.252	0.305	0.296	0.297	0.314	0.277	0.037	0.930	0.328	0.590	0.531	0.611	0.807

Values are least square means and pooled SEM; *n* = 9 in the 0 DON + con group for parameters in the duodenum, and *n* = 10 for all remaining groups. Significant *P* values (*P* < 0.05) are bold, *P* < 0.10 are italicized.

¹*TLR2*, toll-like receptor 2; *TLR4*, toll-like receptor 4; *NFKB*, nuclear factor kappa B; *IL1B*, interleukin 1B; *IL6*, interleukin 6; *IL8*, interleukin 8; *IL10*, interleukin 10; *TNF*, lipopolysaccharide-induced tumor necrosis factor-α; *TGFBI*, transforming growth factor beta 1; *CAS*, caspase 3.

²*P* values for orthogonal contrasts to test linear (lin.) and quadratic (quad.) relationships between control feeding and the 3 increasing levels of DON as well as the overall difference between 0 DON and all DON groups (0 vs. DON).

Table 3. Least squares means of electrophysiological data and tissue permeability in distal jejunum of broiler chickens fed diets with increasing levels of deoxynivalenol (DON; 0 or 10 mg DON/kg diet) and with or without oral lipopolysaccharide (LPS) challenge 1 d before sampling

Item ¹	0 DON + con	10 DON + con	10 DON + lps	SEM	<i>P</i> value
<i>I_{sc}</i> , μA/cm ²	-10.94	-6.04	-15.02	4.24	0.339
<i>G_T</i> , mS/cm ²	6.46 ^{ab}	5.36 ^b	9.21 ^a	1.09	0.050

Values are presented as least square means and SEM; *n* = 10. Significant *P* values (*P* < 0.05) are bold, *P* < 0.10 are italicized.

¹*I_{sc}*, short-circuit current; *G_T*, tissue conductance.

or a DON-related enhanced corticosterone stress response (Antonissen et al., 2017). Because DON modulates both cellular and humoral immunity in chickens (Awad et al., 2013), this may decrease performance of the animals due to energy diversion for the immune response (Klasing et al., 1997, 1998). In line with this, the present changes in the duodenal and jejunal expression of tight junction protein genes (*CLDN1*), TLR, and cytokines may have

contributed to the reduced growth of chickens fed DON-contaminated diets (Lucke et al., 2017). The additional oral LPS challenge with highly immunogenic LPS from *E. coli* O55:B5 (Wu et al., 2013), however, barely altered the observed DON-related serum and intestinal inflammatory response but enhanced the jejunal permeability.

Dietary DON is rapidly absorbed and detoxified by sulfation and bacterial de-epoxidation

Table 4. Least squares means of serum concentration α -1-acid glycoprotein (AGP) and IgA in broiler chickens fed diets with increasing levels of deoxynivalenol (DON; 0, 2.5, 5, or 10 mg DON/kg diet) and with or without oral lipopolysaccharide (LPS) challenge 1 day before sampling

Item	no LPS				LPS				Fixed effect, <i>P</i> values				Contrasts, <i>P</i> values ¹		
	DON, mg/kg feed								SEM	DON	LPS	DON $\times$ LPS	0 vs. DON	lin.	quad.
	0	2.5	5	10	0	2.5	5	10							
AGP, μ g/mL	148	163	152	144	160	166	166	162	12.6	0.765	0.183	0.935	0.612	0.840	0.329
IgA, μ g/mL	84	108	89	119	82	70	69	115	16.1	0.090	0.165	0.656	0.351	0.075	0.176

Values are least square means and SEM, $n = 10$ per group. Significant *P* values ($P < 0.05$) are bold, $P < 0.10$ are italicized.

¹*P* values for orthogonal contrasts to test linear (lin.) and quadratic (quad.) relationships between control feeding and the 3 increasing levels of DON as well as the overall difference of 0 DON vs. all DON groups (0 vs. DON).

during small intestinal transit (Yu et al., 2010; Schwartz-Zimmermann et al., 2015). For this reason, the concentration of DON and its metabolites probably declined from the duodenum to the jejunum in the present study. This may explain our finding that, from the target genes that were altered by DON, only the expression of *CLDN1* was similarly up-regulated with increasing DON concentrations in the duodenal and jejunal mucosa in the present study, whereas the other genes showed different responses in both segments. The question remains whether the DON-related changes in the mucosal gene expression were a direct effect of DON or indirectly via an altered activation of pathogen-recognition receptors as indicated by the DON-related changes in expression levels of *TLR2* in both intestinal segments and of *TLR4* in the duodenum.

The main function of the *CLDN1* protein is to prevent the paracellular diffusion of small molecules through tight junctions (Günzel and Yu, 2013; Suzuki, 2013). Consequently, up-regulation of *CLDN1* expression may have been a protective action to limit the paracellular uptake of DON in the present study and hence to alleviate the effect of DON on active and dynamic processes inside cells, particularly in cells exhibiting high-protein activity (Chen et al., 2017). DON has been reported to directly act on eukaryotic ribosomes at the 60S subunit, activate MAPK, and subsequently induce proinflammatory pathways (Pestka, 2007, 2010). Proinflammatory cytokines trigger the expression of tight junction proteins of mucins and claudins, including *CLDN1* (Poritz et al., 2011; Cornick et al., 2015; Garcia-Hernandez et al., 2017). This may be a possible explanation for the similarly greater expression of *IL6*, *CLDN1*, and *MUC1* (as trend) that we observed for the duodenal mucosa of chickens of the DON groups compared with the 0 DON group. However, the increasing dietary DON concentrations did not modify the gene expression levels of the other 3 targeted tight-junction proteins

and *MUC2*. In general, it can be argued that gene expression profiles do not entirely reflect functional protein profiles. Nevertheless, they are the first step and consequently indicate changes in the adaptive response of the mucosa towards the dietary DON. Similarly, DON did not cause changes in the jejunal electrophysiological parameters of the chickens in this study. It needs to be considered though that these parameters were only assessed in chickens of the 10 DON group, which generally showed a less pronounced response than the 5 DON group. This may also explain that we did not find changes in the *CAS3* expression, although increasing DON concentrations have been reported to promote apoptosis in chicken spleen lymphocytes and tissue in vitro (Ren et al., 2015; Chen et al., 2017).

Our findings for the duodenal and jejunal *TLR2* and *TLR4* expression after lifelong exposure to increasing DON concentrations may indicate that part of the present DON effects may have been mediated by DON-related differences in the development of the intestinal microbiota compared with the 0 DON treatment (Lucke et al., 2018). With potentially less DON and more DON metabolites available in the jejunum than in the duodenum, the *TLR2* and *TLR4* expression may possibly reflect changes in the bacterial community structure. Cell wall components of Gram-positive bacteria, such as lipoteichoic acids, peptidoglycan, and lipoproteins, act as ligands for *TLR2* (Tizard, 2009). The up-regulated and down-regulated expressions of *TLR2* in the duodenum and jejunum, respectively, of DON-fed birds may therefore indicate DON-associated alterations in the duodenal and jejunal microbiota. In line with this, many Gram-positive *Firmicutes* bacteria including *Clostridiales*, *Anaerofilum*, *Collinsella*, *Bacillus*, and *Lactobacillus* present in the upper digestive tract of chickens were reported either to de-epoxidize or adsorb DON (Yu et al., 2010; Franco et al., 2011). As for *TLR4*, the immune reactivity of its bacterial ligand LPS

largely depends on its taxonomic identity. In general, *E. coli*-derived LPS commonly evokes a strong immune response via TLR4 and nuclear factor NF- κ B activation (Wyns et al., 2015; Cheng et al., 2017), inducing the expression of other proinflammatory cytokines, mucins, and tight-junction proteins. In the present study, we observed trends for higher duodenal expression of *TLR4* and *NFKB* with DON, which, in turn, may have triggered the higher expression levels of *IL6*, *CLDN1*, and *MUC1* in the duodenum of chickens of the DON-groups.

As regulatory and anti-inflammatory cytokines (Rothwell et al. 2004; Maynard et al., 2012; Bauché and Marie, 2017), the DON-related expression levels of *IL10* and *TGFBI* may support intestinal microbiota-related changes in the mucosal gene expression. For instance, *TGFBI* expression has been related to immunological tolerance towards microbial compounds, including short-chain fatty acids, lactic acid, surface proteins of lactobacilli, peptidoglycan-derived neuropeptides, and histamine (Kashiwagi et al., 2015; Bauché and Marie, 2017; Ihara et al., 2017). Accordingly, decreasing jejunal *IL8* and *IL10* expression might be associated with the DON-related decreased *TLR2* expression and thus with changes in the microbiota composition (Cotton et al., 2016; Kaji et al., 2018).

We could not confirm our hypothesis that the additional LPS challenge may potentiate dietary DON effects (Xu et al., 2011). Considering the rapid transit of digesta of ~2 to 7 h in the chicken intestine (Hughes, 2008), LPS effects on the intestinal barrier might already have disappeared at the time point of sampling. In fact, LPS is detoxified in the intestinal lumen via the action of bile salts and alkaline phosphatase in the intestinal lumen and liver (Mani et al., 2012; Guerville and Boudry, 2016). Nevertheless, the enhanced duodenal expression of *OCN* and *TNF* may still indicate an upregulation of proinflammatory pathways caused by the single oral LPS challenge. In contrast to that, results for the jejunal tissue conductance suggested an increased mucosal permeability in chickens of the 10 DON + LPS group, which may involve altered expression of other tight junction proteins, such as pore-forming claudins (Garcia-Hernandez et al., 2017). Moreover, LPS was shown to induce changes in small intestinal structure, such as decreasing villus height (Hu et al., 2011; Zhang et al., 2013; Ghareeb et al., 2016), which may lead to observed increases in tissue conductance.

In conclusion, the present data show the small intestinal site-related effects of DON on the expression of genes for tight junction proteins and

proinflammatory signaling pathways, which may be associated with the rapid metabolism and detoxification of DON in the upper digestive tract. The reduced DMI of chickens receiving the diets with higher DON contamination levels may explain why we did not observe clear dose-response relationship between the mucosal gene expression and the increasing DON concentrations. This might be related to a generally enhanced inflammatory response as indicated by the higher serum IgA in chickens of the 10 DON group. The additional oral LPS challenge did not markedly potentiate the DON effect but it appeared to induce a certain proinflammatory response in the duodenum and enhanced the mucosal permeability in the jejunum.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Animal Science* online.

LITERATURE CITED

- Andersen, C. L., J. L. Jensen, and T. F. Ørntoft. 2004. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Res.* 64:5245–5250. doi:10.1158/0008-5472.CAN-04-0496
- Andretta, I., M. Kipper, C. R. Lehen, L. Hauschild, M. M. Vale, and P. A. Lovatto. 2011. Meta-analytical study of productive and nutritional interactions of mycotoxins in broilers. *Poult. Sci.* 90:1934–1940. doi:10.3382/ps.2011-01470
- Antonissen, G., S. De Baere, M. Devreese, F. Van Immerseel, A. Martel, and S. Croubels. 2017. Feed contamination with *fusarium* mycotoxins induces a corticosterone stress response in broiler chickens. *Poult. Sci.* 96:14–17. doi:10.3382/ps/pew280
- Antonissen, G., F. Van Immerseel, F. Pasmans, R. Ducatelle, F. Haesebrouck, L. Timbermont, M. Verlinden, G. P. Janssens, V. Eeckhout, M. Eeckhout, et al. 2014. The mycotoxin deoxynivalenol predisposes for the development of *clostridium perfringens*-induced necrotic enteritis in broiler chickens. *PLoS One* 9:e108775. doi:10.1371/journal.pone.0108775
- Antonissen, G., F. Van Immerseel, F. Pasmans, R. Ducatelle, G. P. Janssens, S. De Baere, K. C. Mountzouris, S. Su, E. A. Wong, B. De Meulenaer, et al. 2015. Mycotoxins deoxynivalenol and fumonisins alter the extrinsic component of intestinal barrier in broiler chickens. *J. Agric. Food Chem.* 63:10846–10855. doi:10.1021/acs.jafc.5b04119
- Aviagen. 2014. Ross broiler management handbook. http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-Broiler-Handbook-2014i-EN.pdf (Accessed 15 December 2016).
- Awad, W. A., M. Hess, M. Twarużek, J. Grajewski, R. Kosicki, J. Böhm, and J. Zentek. 2011. The impact of the *fusarium* mycotoxin deoxynivalenol on the health and performance

- of broiler chickens. *Int. J. Mol. Sci.* 12:7996–8012. doi:10.3390/ijms12117996
- Awad, W., K. Ghareeb, J. Böhm, and J. Zentek. 2013. The toxicological impacts of the fusarium mycotoxin, deoxynivalenol, in poultry flocks with special reference to immunotoxicity. *Toxins (Basel)*. 5:912–925. doi:10.3390/toxins5050912
- Bauché, D., and J. C. Marie. 2017. Transforming growth factor β : a master regulator of the gut microbiota and immune cell interactions. *Clin. Transl. Immunology* 6:e136. doi:10.1038/cti.2017.9
- Chen, S. S., Y. H. Li, and M. F. Lin. 2017. Chronic exposure to the *Fusarium* mycotoxin deoxynivalenol: impact on performance, immune organ, and intestinal integrity of slow-growing chickens. *Toxins (Basel)*. 9:334. doi:10.3390/toxins9100334
- Cheng, P., T. Wang, W. Li, I. Muhammad, H. Wang, X. Sun, Y. Yang, J. Li, T. Xiao, and X. Zhang. 2017. Baicalin alleviates lipopolysaccharide-induced liver inflammation in chicken by suppressing TLR4-mediated NF- κ B pathway. *Front. Pharmacol.* 8:547. doi:10.3389/fphar.2017.00547
- Cornick, S., A. Tawiah, and K. Chadee. 2015. Roles and regulation of the mucus barrier in the gut. *Tissue Barriers*. 3:e982426. doi:10.4161/21688370.2014.982426
- Cotton, J. A., J. M. Platnich, D. A. Muruve, H. B. Jijon, A. G. Buret, and P. L. Beck. 2016. Interleukin-8 in gastrointestinal inflammation and malignancy: induction and clinical consequences. *Int. J. Interf. Cytokine Mediat. Res.* 8:13–34. doi:10.2147/IJICMR.S63682
- EFSA. 2013. Deoxynivalenol in food and feed: occurrence and exposure. *EFSA J.* 11:3379. doi:10.2903/j.efsa.2013.3379
- Franco, T. S., S. Garcia, E. Y. Hirooka, Y. S. Ono, and J. S. dos Santos. 2011. Lactic acid bacteria in the inhibition of *Fusarium graminearum* and deoxynivalenol detoxification. *J. Appl. Microbiol.* 111:739–748. doi:10.1111/j.1365-2672.2011.05074.x
- Ganey, P. E., and R. A. Roth. 2001. Concurrent inflammation as a determinant of susceptibility to toxicity from xenobiotic agents. *Toxicology* 169:195–208. doi:10.1016/S0300-483X(01)00523-6
- Garcia-Hernandez, V., M. Quiros, and A. Nusrat. 2017. Intestinal epithelial claudins: expression and regulation in homeostasis and inflammation. *Ann. N. Y. Acad. Sci.* 1397:66–79. doi:10.1111/nyas.13360
- Ghareeb, K., W. A. Awad, J. Böhm, and Q. Zebeli. 2015. Impacts of the feed contaminant deoxynivalenol on the intestine of monogastric animals: poultry and swine. *J. Appl. Toxicol.* 35:327–337. doi:10.1002/jat.3083
- Ghareeb, K., W. A. A. Awad, J. Böhm, and Q. Zebeli. 2016. Impact of luminal and systemic endotoxin exposure on gut function, immune response and performance of chickens. *Worlds. Poult. Sci. J.* 72:367–380. doi:10.1017/S0043933916000180
- Guerville, M., and G. Boudry. 2016. Gastrointestinal and hepatic mechanisms limiting entry and dissemination of lipopolysaccharide into the systemic circulation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 311:G1–G15. doi:10.1152/ajpgi.00098.2016
- Günzel, D., and A. S. Yu. 2013. Claudins and the modulation of tight junction permeability. *Physiol. Rev.* 93:525–569. doi:10.1152/physrev.00019.2012
- Hu, X. F., Y. M. Guo, J. H. Li, G. L. Yan, S. Bun, and B. Y. Huang. 2011. Effects of an early lipopolysaccharide challenge on growth and small intestinal structure and function of broiler chickens. *Can. J. Anim. Sci.* 91:379–384. doi:10.4141/cjas2011-008
- Hughes, R. J. 2008. Relationship between digesta transit time and apparent metabolisable energy value of wheat in chickens. *Br. Poult. Sci.* 49:716–720. doi:10.1080/00071660802449145
- Ihara, S., Y. Hirata, and K. Koike. 2017. TGF- β in inflammatory bowel disease: a key regulator of immune cells, epithelium, and the intestinal microbiota. *J. Gastroenterol.* 52:777–787. doi:10.1007/s00535-017-1350-1
- Kaji, R., J. Kiyoshima-Shibata, S. Tsujibe, M. Nanno, and K. Shida. 2018. Short communication: probiotic induction of interleukin-10 and interleukin-12 production by macrophages is modulated by co-stimulation with microbial components. *J. Dairy Sci.* 101:2838–2841. doi:10.3168/jds.2017-13868
- Kashiwagi, I., R. Morita, T. Schichita, K. Komai, K. Saeki, M. Matsumoto, K. Takeda, M. Nomura, A. Hayashi, T. Kanai, et al. 2015. Smad2 and smad3 inversely regulate TGF- β autoinduction in *Clostridium butyricum*-activated dendritic cells. *Immunity* 43:65–79. doi:10.1016/j.immuni.2015.06.010
- Klasing, K. C. 1998. Avian macrophages: regulators of local and systemic immune responses. *Poult. Sci.* 77:983–989. doi:10.1093/ps/77.7.983
- Klasing, K.C., and D.R. Korver. 1997. Leukocytic cytokines regulate growth rate and composition following activation of the immune system. *J. Anim. Sci.* 75:58–67.
- Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻($\Delta\Delta C_T$) method. *Methods* 25:402–408. doi:10.1006/meth.2001.1262
- Lucke, A., J. Böhm, Q. Zebeli, and B. U. Metzler-Zebeli. 2018. Dietary deoxynivalenol contamination and oral lipopolysaccharide challenge alters the cecal microbiota of broiler chickens. *Front. Microbiol.* 9:804. doi:10.3389/fmicb.2018.0080
- Lucke, A., B. Doupovec, P. Paulsen, Q. Zebeli, and J. Böhm. 2017. Effects of low to moderate levels of deoxynivalenol on feed and water intake, weight gain, and slaughtering traits of broiler chickens. *Mycotoxin Res.* 33:261–271. doi:10.1007/s12550-017-0284-z
- Mani, V., T. E. Weber, L. H. Baumgard, and N. K. Gabler. 2012. Growth and development symposium: endotoxin, inflammation, and intestinal function in livestock. *J. Anim. Sci.* 90:1452–1465. doi:10.2527/jas.2011-4627
- Maynard, C. L., C. O. Elson, R. D. Hatton, and C. T. Weaver. 2012. Reciprocal interactions of the intestinal microbiota and immune system. *Nature* 489:231–241. doi:10.1038/nature11551
- Metzler-Zebeli, B. U., M. Hollmann, J. R. Aschenbach, and Q. Zebeli. 2017. Comparison of electrogenic glucose transport processes and permeability between proximal and distal jejunum of laying hens. *Br. Poult. Sci.* 58:278–282. doi:10.1080/00071668.2017.1280773
- Metzler-Zebeli, B. U., P. G. Lawlor, E. Magowan, and Q. Zebeli. 2018. Interactions between metabolically active bacteria and host gene expression at the cecal mucosa in pigs of diverging feed efficiency. *J. Anim. Sci.* 96:2249–2264. doi:10.1093/jas/sky118
- Newman, M. A., R. M. Petri, D. Grüll, Q. Zebeli, and B. U. Metzler-Zebeli. 2018. Transglycosylated starch modulates the gut microbiome and expression of genes related to lipid synthesis in liver and adipose tissue of pigs. *Front. Microbiol.* 9:224. doi:10.3389/fmicb.2018.00224

- Osselaere, A., R. Santos, V. Hautekiet, P. De Backer, K. Chiers, R. Ducatelle, and S. Croubels. 2013. Deoxynivalenol impairs hepatic and intestinal gene expression of selected oxidative stress, tight junction and inflammation proteins in broiler chickens, but addition of an adsorbing agent shifts the effects to the distal parts of the small intestine. *PLoS One* 8:e69014. doi:10.1371/journal.pone.0069014
- Pestka, J. J. 2007. Deoxynivalenol: toxicity, mechanisms and animal health risks. *Anim. Feed Sci. Technol.* 137:283–298. doi:10.1016/j.anifeedsci.2007.06.006
- Pestka, J. J. 2010. Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. *Arch. Toxicol.* 84:663–679. doi:10.1007/s00204-010-0579-8
- Pfaffl, M. W., A. Tichopad, C. Prgomet, and T. P. Neuvians. 2004. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: bestkeeper–excel-based tool using pairwise correlations. *Biotechnol. Lett.* 26:509–515. doi:10.1023/B:BILE.0000019559.84305.47
- Poritz, L. S., L. R. Harris, III, A. A. Kelly, and W. A. Koltun. 2011. Increase in the tight junction protein claudin-1 in intestinal inflammation. *Dig. Dis. Sci.* 56:2802–2809. doi:10.1007/s10620-011-1688-9
- Ren, Z., Y. Wang, H. Deng, Y. Deng, J. Deng, Z. Zuo, Y. Wang, X. Peng, H. Cui, and L. Shen. 2015. Deoxynivalenol induces apoptosis in chicken splenic lymphocytes via the reactive oxygen species-mediated mitochondrial pathway. *Environ. Toxicol. Pharmacol.* 39:339–346. doi:10.1016/j.etap.2014.11.028
- Rothwell, L., J. R. Young, R. Zoorob, C. A. Whittaker, P. Hesketh, A. Archer, A. L. Smith, and P. Kaiser. 2004. Cloning and characterization of chicken IL-10 and its role in the immune response to *Eimeria maxima*. *J. Immunol.* 173:2675–2682. doi:10.4049/jimmunol.173.4.2675
- Schwartz-Zimmermann, H. E., P. Fruhmann, S. Dänicke, G. Wiesenberger, S. Caha, J. Weber, and F. Berthiller. 2015. Metabolism of deoxynivalenol and deepoxy-deoxynivalenol in broiler chickens, pullets, roosters and turkeys. *Toxins (Basel)*. 7:4706–4729. doi:10.3390/toxins7114706
- Suzuki, T. 2013. Regulation of intestinal epithelial permeability by tight junctions. *Cell. Mol. Life Sci.* 70:631–659. doi:10.1007/s00018-012-1070-x
- Tizard, I. R. 2009. *Veterinary immunology*. 8th ed. Elsevier, St. Louis, MO.
- Wallace, R. J., J. Gropp, N. Dierick, L. G. Costa, G. Martelli, P. G. Brantom, V. Bampidis, D. W. Renshaw, and L. Leng. 2016. Risks associated with endotoxins in feed additives produced by fermentation. *Environ. Health* 15:5. doi:10.1186/s12940-016-0087-2
- Wu, Q. J., Y. M. Zhou, Y. N. Wu, L. L. Zhang, and T. Wang. 2013. The effects of natural and modified clinoptilolite on intestinal barrier function and immune response to LPS in broiler chickens. *Vet. Immunol. Immunopathol.* 153:70–76. doi:10.1016/j.vetimm.2013.02.006
- Wyns, H., E. Plessers, P. De Backer, E. Meyer, and S. Croubels. 2015. In vivo porcine lipopolysaccharide inflammation models to study immunomodulation of drugs. *Vet. Immunol. Immunopathol.* 166:58–69. doi:10.1016/j.vetimm.2015.06.001
- Xu, L., S. D. Eicher, and T. J. Applegate. 2011. Effects of increasing dietary concentrations of corn naturally contaminated with deoxynivalenol on broiler and turkey poult performance and response to lipopolysaccharide. *Poult. Sci.* 90:2766–2774. doi:10.3382/ps.2011-01654
- Yu, H., T. Zhou, J. Gong, C. Young, X. Su, X. Z. Li, H. Zhu, R. Tsao, and R. Yang. 2010. Isolation of deoxynivalenol-transforming bacteria from the chicken intestines using the approach of PCR-DGGE guided microbial selection. *BMC Microbiol.* 10:182. doi:10.1186/1471-2180-10-182
- Zhang, X., L. Zhao, F. Cao, H. Ahmad, G. Wang, and T. Wang. 2013. Effects of feeding fermented ginkgo biloba leaves on small intestinal morphology, absorption, and immunomodulation of early lipopolysaccharide-challenged chicks. *Poult. Sci.* 92:119–130. doi:10.3382/ps.2012-02645