GASTROINTESTINAL BIOLOGY

A multicomponent mycotoxin deactivator modifies the response of the jejunal mucosal and cecal bacterial community to deoxynivalenol contaminated feed and oral lipopolysaccharide challenge in chickens¹

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Abstract: Mycotoxin deactivators are a widely used strategy to abrogate negative effects of mycotoxin-contaminated feed. It has not been adequately evaluated whether these deactivators may detoxify bacterial toxins in the intestinal lumen and subsequently lower the inflammatory response in chickens. The present objective was to study the effect of a multicomponent mycotoxin deactivator (B), containing a bentonite and a bacterial strain capable to enzymatically biotransform trichothecenes especially deoxynivalenol (DON), when supplemented to a DON-contaminated feed in combination with an oral lipopolysaccharide challenge on visceral organ size, expression of innate immune genes and mucosal permeability in the small intestine as well as on the cecal bacterial composition and metabolites in broiler chickens. Eighty 1-d-old male chickens were randomly allotted to four treatment groups in two replicate batches ($n = 10$ /treatment/replicate): 1) basal diet without DON (CON), 2) CON diet supplemented with B (2.5 mg B/kg feed) (CON-B), 3) CON diet contaminated with 10 mg DON/kg feed (DON), and 4) DON diet supplemented with 2.5 mg B/kg feed (DON-B). In half of the chickens per treatment, effects were assessed under nonchallenge conditions, whereas in the other half of birds, to increase their intestinal bacterial toxin load, effects were tested after an oral challenge with 1 mg LPS/kg BW from *Escherichia coli* O55:B5 on the day before sampling. DON reduced ($P < 0.05$) the weight of bursa fabricii and thymus. DON increased the expression level of intestinal alkaline phosphatase at the duodenal mucosa ($P = 0.027$) but did not modify jejunal gene expression and mucosal permeability. The LPS challenge decreased the jejunal MUC2 expression but increased ZO1 and IL6 expression compared to the unchallenged animals ($P < 0.05$). DON $\times$ B interactions indicated lower expression of IL10 in duodenum and NF κ B in jejunum with the B diet but higher expression with the DON-B diet ($P = 0.050$). Furthermore, the B lowered jejunal expression of NF κ B and IL6 but only in LPS-challenged chickens ($P < 0.05$). Alterations in the cecal microbiota composition and VFA profile were likely associated with alterations in host physiology in the small intestine caused by DON, B, and LPS. According to the present data, B appeared to have potential to detoxify antigens other

than DON in the intestinal lumen of chickens, whereby the toxin load may limit the efficacy of B to modify the intestinal and systemic response as indicated by interactions of DON, B, and LPS.

Key words: barrier function, broiler chicken, innate immunity, microbiome, mucosal gene expression, mycotoxin-deactivator

Introduction

Deoxynivalenol (DON) is a type B trichothecene mycotoxin produced by *Fusarium* species, which frequently contaminates cereal grains, thereby negatively affecting performance and health of animals when ingested even at low concentrations (EFSA, 2013). Once trichothecene mycotoxins are ingested, they are rapidly absorbed in the small intestine, and exert their toxicities locally on the intestinal epithelium and after absorption systemically (Awad et al., 2013; Gratz et al., 2018). Consumption of DON typically reduces chicken's feed intake while impairing intestinal morphology, nutrient absorption, barrier function, and the innate immune response (Awad et al., 2013; Osselaere et al., 2013; Lucke et al., 2018). By acting as an inhibitor of 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). Microbial metabolism experiments have shown that the chicken intestinal microbiota can metabolize DON compounds, which, in turn, alters the toxicity of metabolites for the host (Schwartz-Zimmermann et al., 2015; Gratz et al., 2018). We could recently show that DON reduced the diversity of the bacterial microbiota in chicken ceca (Lucke et al., 2018), which is the intestinal site with the majority of microbes and highest fermentation intensity (Broom and Kogut, 2018). Lower bacterial diversity may decrease the stability of the intestinal microbiota (Stanley et al., 2014), rendering the bird more vulnerable to bacterial dysbiosis.

To abrogate the negative effects of DON-contaminated feed on the host, a widely used strategy is the addition of mycotoxin binders and/or deactivators (Jin et al., 2017). Mycotoxin deactivators are capable to transform DON and related trichothecenes to nontoxic metabolites due to enzymatically biotransformation. Mycotoxin binders are typically indigestible adsorbents (e.g., bentonite), which adsorb mycotoxins in the gastrointestinal tract without dissociating, thereby limiting intestinal mycotoxin uptake and alleviating their adverse effects. As the binding sites of these binders are not specific, they may bind other luminal components, such as xenobiotics or dietary compounds. In line with that, results from weaned pigs support binding of bacterial toxins, such as endotoxins, by mycotoxin binders, thereby diminishing the pro-inflammatory innate immune response (Jin et al., 2017). Whether mycotoxin deactivators exert additional anti-inflammatory properties has not been sufficiently elucidated for chickens.

We therefore hypothesized that 1) the addition of a mycotoxin deactivator would alleviate DON-related effects on growth performance, immune organs, and intestinal innate immune response; and 2) if detoxification of other bacterial toxins occurs, the mycotoxin deactivator would diminish the intestinal innate immune response stronger under endotoxin challenge than under non-challenge conditions. Biotransformation of DON and other bacterial toxins by a mycotoxin deactivator may avert bacterial taxonomic alterations at the major site of intestinal fermentation in the ceca.

Therefore, the objective of the present study was to investigate the effect of the dietary addition of a multicomponent mycotoxin deactivator (B) to DON-contaminated feed on performance, visceral organ size, expression of innate immune genes, and barrier function in the small intestine as well as on the cecal bacterial taxonomic composition and metabolites in broiler chickens. In choosing a multifactorial experimental design, the effects of DON and B were thereby assessed under nonchallenge and challenge conditions, after administering *Escherichia coli* O55:B5-lipopolysaccharide (LPS) orally to increase the intestinal load of highly immune stimulating endotoxin.

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).

Animals, Diets, and Experimental Design

Eighty-one-day-old male broiler chicks (ROSS308) were purchased from a commercial hatchery (Brüterei Schlierbach GmbH, Pettenbach, Austria) and used in a completely randomized 2 (two DON levels: 0 or 10 mg/kg feed) × 2 (two B levels: 0 or 2.5 mg/kg feed) × 2 (two LPS levels: 0 or 1 mg LPS/kg BW) factorial design with two replicate batches. Chicks were housed in flatdeck cages (0.36 m² each) in groups of four animals per cage from week 1 to 3 of the experiment and two birds per cage for the remaining time of the experiment. Housing and lighting were similar to Lucke et al. (2017, 2018). Birds were spray-vaccinated against infectious bronchitis (Poultvac IB Primer, Zoetis, Berlin, Germany) at the hatchery and again orally on day 12 of life (Nobilis IB 4–91, MSD Animal Health, Intervet International, Boxmeer, The Netherlands).

Chicks were randomly allocated to four feeding groups between the first day of life and 5 weeks of age: 1) basal diet without DON (CON), 2) CON diet supplemented with B (CON-B; 2.5 mg B/kg feed), 3) CON diet with 10 mg DON/kg feed (DON), and 4) the DON diet supplemented with 2.5 mg B/kg feed (DON-B). The used product was a commercially available product (Mycofix, Biomin, Herzogenburg, Austria), containing a bentonite and a bacterial strain capable to enzymatically biotransform trichothecenes especially DON, registered in the EU under the category 1 m substances for reduction of the contamination of feed by mycotoxins (aflatoxin B1) (EU Regulation No. 1060/2013). Birds had free access to feed and water, provided in one bell feeder and drinker per cage, respectively, throughout the experimental period. The basal diet consisted mainly of wheat and soybean meal (Supplementary Table S1), supplemented with a commercial mineral-vitamin supplement (Biomin BR 15% CAN,

Biomim GmbH, Herzogenburg, Austria) and was prepared before the start of the experiment for both experimental batches. The experimentally contaminated diets were prepared by first mixing a premix, consisting of 0.05% DON culture material and 99.95% of the basal diet. This premix was then mixed at an inclusion rate of 1% into the final diet. For the B-containing diets, a premix consisting of 750 g of the basal diet and 250 g B were prepared and mixed at an inclusion rate of 1% into the diet resulting in a final concentration of 2.5 mg B/kg diet. After mixing the diets, several subsamples from each diet were taken, thoroughly mixed and used for analysis. In addition, feed samples for dry matter (DM) content analysis were collected in experimental week 3 in both replicate batches. Proximate nutrient analysis, including DM, crude protein, crude fat, crude ash, crude fiber, starch and sugar, was performed according to VDLUFA methods (Naumann and Basler, 2012) before the start of the experiment. Likewise, diets were analyzed for DON concentrations using the Spectrum 380 Multi-Mycotoxin-Analysis, which measures more than 380 mycotoxins and their metabolites and is based on HPLC-MS/MS (Romer Labs Diagnostic GmbH; Tulln, Austria) before the start of the experiment.

Measurements and Sample Collection

The feed intake per bird was estimated daily by measuring the weight difference of the feeder compared to the previous day (Lucke et al., 2017). The used feeder type largely prevented feed spillage. However, if feed spills occurred, they were collected from the cardboard paper covering the floor of each cage. Initial body weight (BW) and BW at slaughter were used to calculate the average daily weight gain per chicken for the entire experimental period. Moreover, chicken's health was monitored daily by visual inspection throughout the whole experimental period.

Due to the Ussing chamber experiment, organ samples were collected from experimental days 33–37. One day before sample collection, half of the birds within each feeding group received a dose of 1 mg LPS/kg BW (LPS L2880 from *Escherichia coli* O55:B5, Sigma-Aldrich, St. Louis, MO) by crop gavage, whereas the other half of the birds received sterile distilled water as control treatment. The next day, chickens were euthanized by an overdose of Thiopental (50–100 mg/kg BW; Thiopental medicamentum pharma GmbH, Allerheiligen im Mürztal, Austria) into the wing vein and subsequent exsanguination. The whole gastrointestinal tract was carefully removed and the respective segments from the small intestine for the gene expression and Ussing chamber experiments as well as digesta from both ceca were collected. For the gene expression experiment, similar to Lucke et al. (2018), the duodenum from the pylorus to the end of the pancreatic loop and the proximal part of the jejunum (30 cm taken from the beginning of the jejunum) were excised, opened longitudinally at the mesenteric border, cleaned with sterile, ice-cold phosphate-buffered saline and carefully blotted dry with paper towel. Thereafter, the mucosa was scraped using a glass microscopic slide and immediately snap-frozen in liquid nitrogen and stored at -80°C until analysis. In addition, a 20 cm-tube piece was taken distal from the Meckel's diverticulum for the gut electrophysiological measurements, immediately transferred into ice-cold transport buffer (Lucke et al., 2018) and gassed with carbogen gas (95% O_2 and 5% CO_2) for transport to the Ussing chamber laboratory. Ceca were opened and the total content was collected, homogenized and aliquots were snap-frozen in liquid nitrogen and stored at -80°C for the microbiome analysis and at -20°C for the analysis

of microbial metabolites. All intestinal samples were collected and processed within 20 min after death of the animal.

RNA Isolation, Complementary DNA Synthesis, and Quantitative PCR

The RNA isolation from 20 mg duodenal and jejunal scrapings, complementary DNA (cDNA) synthesis and quantitative PCR (qPCR) were performed similarly as described in Lucke et al. (2018) 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 using the Turbo DNA kit (Life Technologies Limited, Vienna, Austria). The 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), with RNA integrity numbers (RIN) ranging from 8.0 to 10.0. Afterwards, 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 (Lucke et al., 2018).

Primer sets for genes targeting the intestinal barrier function and cytokine signaling were previously published (Lucke et al., 2018). Amplification conditions can be found in Lucke et al. (2018). Dissociation curve analysis was performed to test specificity. Negative template controls and RT minus controls using RNA were run on each plate. From the five housekeeping genes [HKG; beta-actin (ACTB), beta-2-microglobulin (B2M), hypoxanthine phosphoribosyltransferase 1 (HPRT1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and small nuclear ribonucleoprotein D3 polypeptide (SNRPD3)], the two most stably expressed HKG (B2M, GAPDH) were determined using NormFinder and BestKeeper as described previously (Lucke et al., 2018; Metzler-Zebeli et al., 2018). The geometric mean expression level of those two HKG was used to calculate the target gene expression according to the $2^{-\Delta\Delta C_t}$ method (Lucke et al., 2018; Metzler-Zebeli et al., 2018). 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. To determine the efficiency $[(10^{(-1/\text{slope})} - 1) \times 100]$ of the single quantitative PCR assays, 5-fold serial dilutions of pooled cDNA samples were performed (Lucke et al., 2018).

Gut Electrophysiology

Gut electrophysiological parameters were evaluated for the distal jejunum of chickens from the CON, DON, and DON-B groups, similar to Lucke et al. (2018). The jejunal preparations were used in Ussing chambers (diameter = 0.91 cm^2), with four successional replicate samples from the jejunal tube piece being evaluated in parallel. The tissues were equilibrated for a period of 20 min under open-circuit conditions before 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 (Mussler Scientific Instruments, Aachen, Germany). At the end of the experiment (185 min), theophylline (final concentration 8 mmol/L in chamber half; Sigma-Aldrich) was added to both serosal and mucosal sides of the Ussing chamber, to verify that the tissues were still alive. Fluorescein 5(6)-isothiocyanate (FITC; 389.38 g/mol; Sigma-Aldrich, Schnelldorf, Austria) and horseradish peroxidase (HRP; 44,000 g/mol; Carl Roth GmbH+Co.

KG, Karlsruhe, Germany) were added to final concentrations of 0.1 mM and 1.8 μ M to the mucosal side, respectively, 5 min after the short-circuit to assess the mucosal-to-serosal flux rates (Metzler-Zebeli et al., 2017). The glucose absorptive tissue response was studied by adding glucose to a final concentration of 10 mmol/L to the buffer at the mucosal side at 45 min after short-circuiting the tissue (Metzler-Zebeli et al., 2017). The chemical effect on glucose transporter function was measured by comparing the I_{sc} and R_t for 1 min before glucose was added to the peak current and resistance response of the exposed tissue (ΔI_{sc} and ΔR_t) obtained within 2 min after the addition of glucose.

16S rRNA Gene Sequencing

Total genomic DNA was isolated from 250 to 300 mg cecal digesta with few modifications as described previously (Lucke et al., 2018) using the DNeasyPowerSoil Kit (Qiagen). The FastPrep-24 instrument (MP Biomedicals, Vienna, Austria; 3 times 60 s of homogenization at 6.5 m/s with intermittent cooling on ice) was used for bead-beating. The DNA concentration was measured using the Qubit Fluorometer (Life Technologies, Vienna, Austria) and the Qubit dsDNA HS Assay Kit (Life Technologies).

The 16S rRNA gene PCR targeting the V3-V4 hypervariable region (forward primer: CCTACGGGNGGCWGCAG and reverse primer: GACTACHVGGGTATCTAATCC), library preparation and Illumina MiSeq sequencing were performed by Microsynth AG (Balgach, Switzerland). Nextera libraries were constructed by ligating sequencing adapters and indices onto purified PCR products using the Nextera XT sample preparation kit according to the manufacturer's instruction (Illumina, Inc., San Diego, CA). For this, the KAPA HiFiHotStart PCR Kit (Roche, Baden, Switzerland) was used, which includes a high-fidelity DNA polymerase. Equimolar amounts of each library were pooled and sequenced on an Illumina MiSeq Personal Sequencer using a 250 bp paired-end protocol. The resultant overlapping paired-end reads were de-multiplexed, trimmed of Illumina adaptor residuals using cutadapt (version 1.8.1; <https://cutadapt.readthedocs.org/>) and the overlapping paired-end reads were stitched using Usearch v10.0.240 by Microsynth.

Sequencing data were analyzed using QIIME (version 1.9.1; Lucke et al., 2018). A quality threshold of 20 was used for quality filtering of fastq files. Chimera were removed by the UCHIME method using the 64-bit version of USEARCH and the GOLD database (drive5.com; Edgar, 2010; Edgar et al., 2011). Open-reference operational taxonomic unit (OTU) picking was performed at 97% similarity level using UCLUST (Edgar, 2010) and taxonomy was assigned against the Greengenes database (gg_13_8; http://qiime.org/home_static/dataFiles.html). OTUs with less than 10 sequences were not considered in the analysis. For α -diversity analyses a rarefaction depth of 10,000 was used.

Microbial Metabolites

The concentration of VFA (acetic acid, propionic acid, isobutyric acid, n-butyric acid, isovaleric acid, n-valeric acid, and caproic acid) was measured in cecal digesta using gas chromatography as described in Lucke et al. (2018). Cecal digesta (0.5 g) was mixed with 500 μ L double-distilled water, 200 μ L phosphoric acid (25%), and 300 μ L of an internal standard (4-methylvalerian acid). Samples were vortexed and centrifuged repeatedly until the supernatant became clear. Afterwards, the supernatant was stored at -80°C and analyzed on the gas chromatograph (Shimadzu 2010 Plus with FID, Kyoto, Japan) equipped with a 30 m $\times$ 0.53 mm $\times$ 0.5 μ m capillary column (TR-Wax, ThermoFisher

Scientific, MA), an AOC-20i autoinjector and a 20S autosampler (Shimadzu) and a flame ionization detector (FID; Fisons EL980). Helium was used as carrier gas (flow rate: 2 mL/min) and the temperatures of the injector and detector were set at 170°C and 190°C , respectively. The GC oven program was the following: The starting temperature was set at 65°C and was heated with a heating rate of 15 K/min to 170°C . The program continued with a heating rate of 35 K/min to 190°C and afterwards with a heating rate of 40 K/min to 200°C . The quantification was performed using the internal standard method, by relating the peak areas of the internal standard and individual VFAs in standards and samples. Standards comprised acetic acid (52.46 μ mol/mL), propionic acid (26.81 μ mol/mL), iso-butyric acid (5.38 μ mol/mL), butyric acid (10.87 μ mol/mL), iso-valeric acid (4.53 μ mol/mL), valeric acid (4.59 μ mol/mL), and caproic acid (3.97 μ mol/mL) as well as 4-methylvalerian acid (23.83 μ mol/mL; all Sigma-Aldrich, Vienna, Austria) as internal standard. A response factor (RRF) for each individual VFA from standard peak areas and injected concentrations was calculated and implemented in the calculation of the VFA concentrations for the cecal samples. The average RRF values were 5.1, 2.4, 1.6, 1.6, 1.2, 1.2, and 1.0 for acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, and caproic acid, respectively. Limits of detection were 267, 111, 33, 52, 24, 24, and 24 nmol/mL for acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, and caproic acid, respectively. Standards and samples were analyzed in the following order. The run started with two standards, then three samples in duplicate were measured which were followed by two standards. If sample replicates surpassed a coefficient of variation of 10%, VFA were newly extracted from digesta and analyzed.

Statistical Analysis

The Shapiro-Wilk test (SAS version 9.4; SAS Institute, Inc., Cary, NC) was used to analyze data of relative bacterial abundances (relative abundance > 0.01%), VFA, gene expression, and gut electrophysiology for normality. The ANOVA was performed using the MIXED procedure of SAS. With the exception of the gut electrophysiological data, DON, B, LPS, as well as their two- and three-way interactions were considered as fixed effects. For gut electrophysiological data, a general treatment effect was considered as fixed effect as only the CON, DON, and DON-B groups were compared. Replicate batch was included as random effect with chicken nested within day as experimental unit. Variance component was used as covariance structure. Degrees of freedom were approximated using the Kenward-Rogers method (ddfm = kr). Results are presented as least squares means and the standard error of the mean (SEM). Pairwise comparisons among least squares means were performed using the probability of difference (pdiff) option in SAS. Significance was declared if $P \leq 0.05$ and a trend was considered if $P \leq 0.10$. Sparse partial least squares (sPLS) regression and relevance network analysis were performed using the package "mixOmics" in R (Rohart et al., 2017) to integrate data of OTUs with the results for VFA.

RESULTS

Dietary DON Contamination and Bird Performance

Analyzed DON concentrations were 0.069 ± 0.016 mg DON/kg feed for CON and CON-B diets, and 11.693 ± 1.169 and 13.206 ± 1.321 mg DON/kg feed for the DON and DON-B diets, respectively. Other DON derivatives (3-acetyl-DON, 15-acetyl-DON, and DON-3-glucoside) in

the basal diet were below the level of detection. Irrespective of the treatment group, chickens remained clinically healthy throughout the study. Likewise, DM intake and growth performance was similar across all treatment groups (Table 1).

Effect on Organ Size, Gut Electrophysiology, and Gene Expression

Dietary DON reduced ($P < 0.05$) the weight of the immune organs bursa fabricii and thymus (Table 1), whereas the B enlarged the spleen but only in chickens without the additional LPS challenge according to the DON $\times$ LPS interaction ($P = 0.009$). The LPS challenge modified the weight of the liver but differently when DON and B were present in the diet ($P = 0.016$).

Effects of DON, B and LPS differed for the duodenal and jejunal mucosa. DON only increased the expression level of intestinal alkaline phosphatase (IAP) at the duodenal mucosa ($P < 0.05$), whereas it did not affect the expression of the investigated target genes at the jejunal mucosa (Tables 2 and 3). There were DON $\times$ B interactions for expression levels of MUC1 in duodenum ($P = 0.068$), IL10 in duodenum ($P = 0.050$) and jejunum ($P = 0.076$), and NFKB in jejunum ($P = 0.050$), showing that B reduced their expression when added to the noncontaminated diet, whereas it increased their expression when supplemented to the DON diet. Twenty-four hours after the oral LPS challenge, the LPS tended ($P = 0.058$) to lower the expression of IAP at the duodenal mucosa. According to the DON $\times$ LPS interaction ($P < 0.05$) in the duodenum, DON increased the OCLN and TNFA expression in chickens receiving the control challenge but not in birds challenged with LPS. In the jejunum, the LPS challenge produced the greatest changes in the observed mucosal gene expression levels, decreasing the expression of MUC2 ($P < 0.001$) and, as trend ($P < 0.10$), of TNFA but increasing that of ZO1 and IL6 ($P < 0.05$), and, as trends ($P < 0.10$), of TLR4 and NFKB. Moreover, the LPS challenge modified the jejunal mucosal response to the B for the expression of NFKB and IL6 as indicated by the B $\times$ LPS interaction ($P < 0.05$).

Albeit DON did not cause a significant effect on the transepithelial resistance of the jejunal mucosa, the addition of B to the DON diet tended ($P = 0.085$) to increase the negative net charge transfer across the jejunal tissue as response to the mucosal glucose addition compared to chickens fed the DON diet alone (Table 4).

Effect on Bacterial Composition and VFA in Cecal Digesta

After quality control and chimera check, a total of 6,776,632 sequencing reads with a mean of 84,708 sequences per sample were obtained for the 80 cecal digesta samples (mean read length 407 bp). Across all treatments, the phyla *Firmicutes* (88.6%) and *Bacteroidetes* (10.2%) dominated in cecal digesta which were mainly represented by the families *Lachnospiraceae* (32.0%), unassigned *Clostridiales*1 (24.5%), *Ruminococcaceae* (16.0%), and *Bacteroidaceae* (10.2%; Table 5).

Bray-Curtis-derived dissimilarity matrices (PERMANOVA) for the cecal bacterial composition indicated that the dietary DON contamination marginally affected the bacterial community structure ($P = 0.094$; data not shown), whereas the diversity (Simpson and Shannon) and species richness (Chao1) were not affected ($P > 0.10$) by treatments (Table 5). Taxonomic assessment of the bacterial microbiota in cecal digesta showed that the dietary DON contamination mainly decreased ($P < 0.05$) the dominant family *Ruminococcaceae* by 12.3% and at genus level, one unassigned *Ruminococcaceae* genus 1 and the *Lachnospiraceae* genus *Dorea* by 16.7% and 21.3%, respectively,

Table 1. Least squares means of dry matter intake, growth, and visceral organ size in broiler chickens fed diets with DON and mycotoxin deactivator (B) and with or without oral LPS challenge 1 d before sampling¹

Item	No LPS				LPS				P-values							
	CON	CON-B	DON	DON-B	CON	CON-B	DON	DON-B	SEM	DON	B	LPS	DON × B	DON × LPS	B × LPS	DON × B × LPS
Dry matter intake, g	77.7	78.8	77.5	75.2	78.6	78.1	78.0	76.1	1.55	0.1458	0.411	0.696	0.285	0.774	0.792	0.666
Average daily gain, g	49.8	51.8	51.9	49.5	50.5	52.4	52.0	52.4	1.70	0.7945	0.699	0.365	0.211	0.717	0.582	0.544
Duodenum, cm/kg BW	12.9	13.0	13.1	14.2	12.8	13.1	13.0	12.8	0.61	0.478	0.499	0.375	0.793	0.343	0.512	0.431
Jejunum, cm/kg BW	48.7	49.5	50.6	53.4	49.8	45.2	48.7	47.2	2.42	0.328	0.718	0.105	0.469	0.483	0.160	0.870
Ileum, cm/kg BW	13.2	13.0	12.0	12.7	13.0	12.2	12.0	12.5	0.82	0.302	0.965	0.612	0.370	0.704	0.711	0.896
Colon, cm/kg BW	4.9	4.7	4.6	5.0	4.9	4.7	4.8	4.5	0.24	0.632	0.639	0.816	0.524	0.674	0.332	0.244
Cecum, cm/kg BW	8.7	9.0	8.5	9.1	9.3	8.6	9.0	9.2	0.44	0.928	0.680	0.485	0.309	0.820	0.251	0.572
Gizzard, g/kg BW	12.0	12.2	12.4	12.9	12.4	12.2	13.0	12.6	0.54	0.162	0.939	0.619	0.970	1.000	0.421	0.766
Proventriculus, g/kg BW	3.3	3.3	3.3	3.3	3.5	3.3	3.2	3.3	0.13	0.583	0.600	0.792	0.516	0.593	0.867	0.620
Liver, g/kg BW	19.2	18.8	18.6	19.6	17.7	18.6	18.8	18.2	0.43	0.474	0.557	0.019	0.923	0.732	0.839	0.016
Pancreas, g/kg BW	2.1	2.1	2.0	2.2	2.1	2.1	2.1	2.0	0.10	0.699	0.454	0.802	0.808	0.876	0.157	0.258
Heart, g/kg BW	5.0	5.2	5.4	5.2	5.1	5.2	5.0	5.6	0.19	0.236	0.192	0.652	0.682	0.888	0.136	0.074
Kidney, g/kg BW	3.0	3.0	2.9	2.9	2.9	3.0	2.8	2.9	0.09	0.373	0.782	0.789	0.854	0.686	0.490	0.798
Bursa fabricii, g/kg BW	2.5	2.6	2.1	2.1	2.4	2.3	2.1	2.0	0.17	0.005	0.781	0.231	0.993	0.588	0.641	0.776
Thymus, g/kg BW	2.7	2.9	2.4	2.7	2.9	2.8	2.5	2.5	0.17	0.028	0.346	0.948	0.574	0.815	0.246	0.995
Spleen, g/kg BW	1.1	1.2	1.0	1.1	1.0	0.9	1.1	1.2	0.06	0.527	0.050	0.148	0.346	0.009	0.206	0.514

¹CON, control diet. Values are least square means and pooled SEM; $n = 10$.

Table 2. Least squares means of relative expression of genes related to barrier function in duodenum and jejunum of broiler chickens fed diets with DON and mycotoxin deactivator (B) and with or without oral LPS challenge 1 d before sampling^a

Gene of interest	No LPS				LPS				P-values							
	CON	CON-B	DON	DON-B	CON	CON-B	DON	DON-B	SEM	DON	B	LPS	DON × B	DON × LPS	B × LPS	DON × B × LPS
Duodenum																
IAP	0.350	0.351	0.525	0.380	0.324	0.286	0.353	0.372	0.0498	0.027	0.252	0.058	0.530	0.530	0.378	0.153
MUC1	0.068	0.051	0.089	0.093	0.091	0.059	0.057	0.197	0.0368	0.112	0.359	0.327	0.068	0.700	0.252	0.150
MUC2	0.400	0.182	0.388	0.333	0.247	0.251	0.265	0.376	0.0757	0.247	0.376	0.357	0.270	0.893	0.101	0.681
CLDN1	0.425	0.361	0.530	0.433	0.448	0.427	0.391	0.524	0.0573	0.185	0.771	0.800	0.457	0.396	0.096	0.254
CLDN5	0.328	0.292	0.424	0.396	0.453	0.424	0.371	0.435	0.0827	0.581	0.901	0.306	0.667	0.250	0.674	0.717
OCLN	0.420	0.352	0.523	0.509	0.453	0.425	0.368	0.428	0.0513	0.224	0.729	0.367	0.330	0.021	0.436	0.824
ZO1	0.417	0.358	0.520	0.403	0.461	0.458	0.413	0.455	0.0571	0.547	0.399	0.583	0.931	0.222	0.186	0.531
Jejunum																
IAP	0.350	0.409	0.382	0.409	0.324	0.323	0.444	0.340	0.0638	0.350	0.911	0.509	0.461	0.566	0.293	0.696
MUC1	0.399	0.180	0.259	0.266	0.336	0.301	0.244	0.210	0.0691	0.230	0.155	0.951	0.251	0.508	0.466	0.258
MUC2	0.464	0.430	0.412	0.415	0.257	0.245	0.340	0.305	0.0587	0.653	0.637	<0.001	0.935	0.208	0.921	0.718
CLDN1	0.708	0.731	0.765	0.721	0.781	0.765	0.706	0.688	0.0436	0.401	0.669	0.902	0.580	0.110	0.920	0.603
CLDN5	0.243	0.298	0.221	0.222	0.369	0.356	0.313	0.255	0.0692	0.199	0.935	0.119	0.614	0.765	0.520	0.961
OCLN	0.626	0.642	0.655	0.685	0.665	0.669	0.661	0.631	0.0377	0.777	0.860	0.873	0.854	0.289	0.515	0.654
ZO1	0.493	0.616	0.522	0.529	0.649	0.680	0.639	0.589	0.0474	0.242	0.406	0.004	0.145	0.753	0.273	0.793

^aCON, control diet. Values are least square means and pooled SEM; n = 10. Genes of interest: IAP, intestinal alkaline phosphatase; MUC1, mucin 1; MUC2, mucin 2; CLDN1, claudin 1; CLDN5, claudin 5; OCLN, occludin; ZO1, zona occludens 1.

whereas it increased *Coprobacillus* by 30.8% compared to control-fed chickens. Moreover, DON tended ($P < 0.10$) to decrease *Oscillospira*, *Eggerthella*, *Bifidobacterium*, and RF39. While the addition of the B to the diet did not modify taxa abundances at family level, it tended ($P < 0.10$) to decrease the abundance of the genus *Clostridium*. Moreover, B decreased the abundance of the unclassified *Ruminococcaceae* genus 2; however, as indicated by the B × LPS interaction ($P = 0.044$), this was only the case 24 h after the oral LPS challenge. The oral LPS challenge further decreased the *Ruminococcaceae* genus 2 ($P = 0.042$), whereas it tended ($P = 0.066$) to increase *Bifidobacterium*. Also, B effects on the cecal abundances of *Bacteroides*, unclassified *Lachnospiraceae* genus 2, unclassified genera of *Peptostreptococcaceae* and [*Mogibacteriaceae*] were influenced by the oral LPS challenge as indicated by the B × LPS interactions ($P < 0.10$). Likewise, DON × B × LPS interactions for *Lachnospiraceae* and *Leuconostocaceae* and [*Ruminococcus*] showed that bacterial responses to DON in the ceca depended on whether B and LPS or both were administered to the chickens.

Regarding the cecal VFA (Table 6), DON tended ($P < 0.10$) to decrease the cecal valerate concentrations; however, as indicated by the DON × B interaction ($P = 0.045$), only in combination with the noncontaminated diet. A similar interaction was found for isovalerate in cecal content that decreased in birds fed the noncontaminated B diet but not in those fed the DON-B diet ($P < 0.05$). To identify the most influential bacterial OTUs associated with the changes of VFA concentrations in cecal digesta, we performed sPLS regression and relevance networking (Table 7). This analysis revealed two influential OTUs [*Ruminococcaceae*-OTU85 and *Lachnospiraceae*-OTU129] being positively and three OTUs [*Clostridiales*-OTU6, *Lachnospiraceae*-OTU102, and *Ruminococcus*-OTU110] being negatively associated with propionate, as well as six most influential OTUs [two *Clostridiales*-OTUs (OTU4 and OTU131), *Ruminococcus*-OTU82, *Ruminococcaceae*-OTU85 and two *Lachnospiraceae*-OTU (OTU61 and OTU146)] being positively associated with valerate ($|r| > 0.4$; Table 7).

Discussion

This study contributes to our understanding about intestinal DON effects in conjunction with a second immune stimulant and a strategy commonly used to decrease the toxicity of DON in diets for poultry. Though chickens were fed the experimental diets from their first day of life in the present experiment, DON hardly exhibited any effect on the small intestinal innate immune response, which may indicate physiological adaptations to cope with the chronic exposure to DON. Nevertheless, the continuing oral uptake of DON compromised the development of central immune organs, that is, bursa fabricii and thymus, potentially demonstrating the detrimental effect of DON on hematopoiesis and functioning of the immune system (Maresca, 2013). Also, the results showed that the B effect on the small intestine depended on whether the diet was contaminated with DON or not. From this, our data would support that mycotoxin deactivators, like the used multicomponent mycotoxin deactivator, may exert anti-inflammatory effects due to nonspecific biotransformation of luminal antigens. With the DON-contaminated diet, however, no further effect of the B to attenuate the mucosal innate immune response in the duodenum and jejunum could be found. In increasing the luminal provision of toxins temporarily, the LPS challenge partly altered the mucosal response to DON and B in the small intestine. Results further suggested that DON-associated alterations in the cecal microbiota may be linked to indirect effects via modification of the host digestive

Table 3. 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 DON and mycotoxin deactivator (B) and with or without oral LPS challenge 1 day before sampling¹

Gene of interest	No LPS					LPS					P-values					
	CON	CON-B	DON	DON-B	CON	CON-B	DON	DON-B	SEM	DON	B	LPS	DON × B	DON × LPS	B × LPS	DON × B × LPS
Duodenum																
TLR2	0.370	0.354	0.508	0.414	0.379	0.350	0.360	0.333	0.0802	0.482	0.469	0.328	0.741	0.307	0.808	0.727
TLR4	0.363	0.317	0.493	0.394	0.478	0.414	0.378	0.467	0.0702	0.418	0.546	0.391	0.615	0.206	0.395	0.301
CAS3	0.436	0.326	0.496	0.449	0.410	0.371	0.336	0.460	0.0567	0.221	0.652	0.426	0.165	0.297	0.136	0.533
NFKB	0.413	0.339	0.537	0.478	0.509	0.413	0.423	0.481	0.0674	0.202	0.373	0.754	0.378	0.145	0.623	0.471
IL1B	0.140	0.198	0.275	0.201	0.254	0.273	0.287	0.256	0.0649	0.401	0.875	0.167	0.322	0.511	0.980	0.656
IL6	0.353	0.322	0.440	0.303	0.485	0.381	0.333	0.369	0.0649	0.606	0.206	0.421	0.850	0.214	0.587	0.189
IL8	0.147	0.164	0.260	0.187	0.297	0.249	0.186	0.236	0.0609	0.939	0.760	0.230	0.959	0.135	0.734	0.279
IL10	0.164	0.062	0.095	0.330	0.205	0.132	0.196	0.526	0.1299	0.119	0.297	0.274	0.050	0.616	0.739	0.857
TGF1B	0.297	0.282	0.402	0.386	0.318	0.322	0.290	0.437	0.0645	0.109	0.514	0.994	0.435	0.505	0.321	0.436
TNFA	0.477	0.345	0.569	0.515	0.428	0.406	0.352	0.407	0.0589	0.267	0.362	0.065	0.357	0.048	0.195	0.994
Jejunum																
TLR2	0.294	0.346	0.344	0.310	0.408	0.342	0.324	0.228	0.0796	0.413	0.527	0.971	0.607	0.348	0.424	0.806
TLR4	0.348	0.373	0.282	0.344	0.516	0.357	0.449	0.378	0.0682	0.468	0.460	0.071	0.528	0.802	0.105	0.792
CAS3	0.517	0.555	0.471	0.542	0.495	0.529	0.550	0.485	0.0447	0.710	0.541	0.828	0.602	0.582	0.267	0.301
NFKB	0.440	0.464	0.330	0.445	0.622	0.369	0.503	0.495	0.0597	0.464	0.473	0.070	0.050	0.424	0.021	0.365
IL1B	0.366	0.377	0.382	0.405	0.507	0.450	0.492	0.327	0.0885	0.703	0.456	0.329	0.703	0.474	0.310	0.635
IL6	0.286	0.348	0.313	0.371	0.509	0.405	0.422	0.331	0.0533	0.463	0.621	0.023	0.954	0.170	0.041	0.916
IL8	0.334	0.220	0.218	0.262	0.294	0.215	0.282	0.201	0.0711	0.622	0.256	0.838	0.443	0.812	0.655	0.429
IL10	0.325	0.064	0.238	0.224	0.236	0.127	0.204	0.232	0.0750	0.495	0.099	0.805	0.076	0.998	0.367	0.605
TGF1B	0.378	0.354	0.439	0.451	0.402	0.429	0.397	0.344	0.0625	0.700	0.832	0.777	0.808	0.164	0.935	0.515
TNFA	0.786	0.778	0.825	0.796	0.711	0.784	0.747	0.776	0.0350	0.406	0.514	0.096	0.519	0.766	0.162	0.817

¹CON, control diet. Values are least square means and pooled SEM; n = 10. Genes of interest: TLR2, toll-like receptor 2; TLR4, toll-like receptor 4; CAS, caspase 3; NFKB, nuclear factor kappa B; IL1B, interleukin 1s; IL6, interleukin 6; IL8, interleukin 8; IL10, interleukin 10; TGF1B, transforming growth factor beta 1; TNFA, lipopolysaccharide-induced tumor necrosis factor-alpha.

Table 4. Least squares means of on basal short-circuit current (I_{sc}), transepithelial resistance (R_t), and electrophysiological responses to glucose addition of the isolated mucosa from the distal jejunum of broiler chickens fed diets with DON or DON and mycotoxin deactivator (B)¹

Item	CON	DON	DON-B	SEM	P-values
Basal electrophysiology					
I_{sc} , $\mu A/cm^2$	-1.77	1.17	-0.57	3.671	0.852
R_t , $\Omega \times cm^2$	248	239	238	22.4	0.949
Glucose response ²					
ΔI_{sc} , $\mu A/cm^2$	-1.50 ^{ab}	-1.09 ^a	-2.67 ^b	0.498	0.085
ΔR_t , $\Omega \times cm^2$	-3.34	-2.98	-3.48	0.559	0.810

¹CON, control diet. Values are least square means and pooled SEM; $n = 10$.

²Glucose addition to a final chamber concentration of 5 mmol/L; ΔI_{sc} is the difference between the maximal I_{sc} value obtained from 2 min after glucose addition and the basal value determined 1 min before glucose addition; ΔR_t is the difference between the basal R_t 1 min before glucose addition and the R_t value obtained from 2 min after glucose addition.

^{a,b}Least square means with no common superscripts differ significantly between groups.

and mucosal physiology and probably lesser to microbial metabolism of DON in the ceca (Gratz et al., 2018). Likewise, it may be assumed that LPS-associated changes in intestinal physiology may be behind bacterial alterations in cecal digesta after the LPS challenge in the present study.

Despite the fact that the present DON contamination was two times as high as the guidance value of the European Union for complete feed for poultry of 5 mg DON/kg feed (2006/576/EC, 2006), chickens in the present study did not show reduced feed intake or growth when exposed to 10 mg DON/kg feed, contrasting previous findings from our group and others (Lucke et al., 2017; Awad et al., 2019). Due to its action as protein synthesis inhibitor (De Walle et al., 2010), DON may have depressed the growth of the birds by impairing the functioning of highly metabolically active organs, including the intestine, liver, and immune organs. Indeed, DON decreased the size of bursa fabricii and thymus in the present study. One feasible explanation for the lack of an effect on performance is the chronic exposure of the animals to the high DON level from the first day of life, which probably promoted DON degradation and detoxification in host tissues (e.g., intestine, liver, and kidneys) and by intestinal bacteria (Schwartz-Zimmermann et al., 2015; Gratz et al., 2018). Since we did not measure DON and DON-metabolites in intestinal digesta or excreta, however, this remains speculative.

Previously, it has been shown that DON is more slowly absorbed in chickens compared with pigs (Dänicke and Brezina, 2013; Broekaert et al., 2017), explaining the greater toxicological effects often observed in pigs after DON exposure compared with chickens. Due to this, we had assumed to find effects on the intestinal mucosa; however, DON had hardly affected the duodenal and jejunal mucosa, except the raised duodenal IAP expression, in the present study. Intestinal alkaline phosphatase plays a role in detoxification of luminal antigens via dephosphorylation and downregulation of inflammatory signaling at the intestinal mucosa (Lallès, 2016). Since DON and DON-metabolites do not carry terminal phosphate groups, the anti-oxidative and anti-inflammatory action of this brush border enzyme may have protected the mucosal integrity against DON in the present study. This may be supported by equal expression

of tight-junction protein genes at the duodenal and jejunal mucosa and jejunal electrophysiology, contrasting previous findings (Awad et al., 2019).

As mentioned above, the maximum biotransformation capacity of B may have limited the efficacy of B with the DON diet to reduce the toxicity of other luminal antigens and to diminish the mucosal innate immune response; potentially explaining the opposite effects on small intestinal expression of *IL10*, *MUC1*, and *NFKB* with CON-B and DON-B diets in the present study. Nevertheless, the increased expression of the anti-inflammatory cytokine *IL10* with B when the chicken was exposed to DON may have limited the proinflammatory response to DON, which may be advantageous for the animal to abrogate an overshooting inflammatory response to DON. Similarly, the higher expression of *MUC1* at the duodenal mucosa when B was added to the DON-contaminated diet may have been beneficial for mucosal barrier function, counteracting potential negative effects of DON on mucin production (Pinton et al., 2015). The greater jejunal negative net charge transfer (ΔI_{sc}) after glucose addition in the Ussing chamber experiment for chickens fed the DON-B diet compared to birds fed the DON diet was indicative for lower sodium-dependent transcellular glucose absorption. Albeit this observation may be indicative for lower nutrient absorption due to a thicker mucus layer, it supports an enforced mucosal barrier due to B, which may be advantageous for the bird when exposed to DON. For the interpretation of this result, it needs to be considered that only the jejunal explant was used in the Ussing chamber without renewed addition of B and DON to the mucosa. Therefore, this change in mucosal functioning can be seen as no immediate reaction to the B and DON but a long-term adaptation to the mycotoxin and the DON deactivator. This may have lowered the vulnerability of the birds to enteric infection and bacterial translocation after DON exposure (Pinton et al., 2015; Awad et al., 2019). In being nonabsorbable and with the main site of action in the intestinal lumen, the enlarging effect that the B had on the spleen was very probably an indirect response, possibly related to alterations in the intestinal immune response and mucosal DON uptake. The enlargement of the spleen as an important secondary lymphoid organ by serving as a reservoir of B lymphocytes and for filtering of blood and destruction of erythrocytes and antigens (Schat et al., 2014) may have thereby compensated for the reduced size of bursa fabricii and thymus and hence may have been advantageous for birds exposed to the dietary DON.

Twenty-four hours after the oral LPS challenge, expression levels of genes within the TLR-4-NF- κ B signal transduction pathway at the jejunal mucosa were upregulated, indicating longer lasting physiological effects on mucosal functioning. Notably, LPS effects were more obvious at the jejunal mucosa than at the duodenal mucosa, which might be related to differences in transit time, mucus production and distribution of Peyer's patches between the two intestinal segments. LPS is predominantly recognized by the TLR-4 (Doyle and O'Neill, 2006) that after activation induces NF- κ B signaling, which then stimulates the release of downstream proinflammatory cytokines and leads to an enforced mucosal barrier via tightening of the tight junctions. Correspondingly, we found an upregulated expression of *TLR4*, *NFKB*, *IL6*, and *ZO1* at the jejunal mucosa after the oral LPS challenge. Contrarily, the LPS challenge downregulated the *MUC2* expression, which may be linked to the origin of the administered LPS because *E. coli* is known for its mucin-attaching and -degrading capabilities. More specifically, the 3-O-sulfo-galactosyl moiety on host mucin acts

Table 5. Least squares means of α -diversity and relative bacterial abundance of selected bacterial taxa in cecal digesta of broiler chickens fed diets with DON and mycotoxin deactivator (B) and with or without oral LPS challenge 1 d before sampling¹

Item ^{2,3}	No LPS				LPS				P-values							
	CON	CON-B	DON	DON-B	CON	CON-B	DON	DON-B	SEM	DON	B	LPS	DON × B	DON × LPS	B × LPS	DON × B × LPS
α-diversity metrics	6.15	6.43	6.24	6.30	6.34	6.40	6.18	6.18	0.118	0.217	0.236	0.960	0.418	0.297	0.387	0.646
	0.94	0.96	0.95	0.95	0.96	0.96	0.95	0.95	0.012	0.648	0.248	0.331	0.697	0.712	0.378	0.733
	1517	1417	1384	1488	1411	1450	1345	1389	67.527	0.325	0.654	0.272	0.278	0.730	0.684	0.303
Family																
f_Lachnospiraceae	31.7	34.8	32.1	29.2	33.5	31.6	29.7	33.1	1.598	0.102	0.715	0.985	0.878	0.495	0.770	0.015
f_Ruminococcaceae	16.9	17.3	14.3	16.2	17.7	16.4	15.2	14.2	0.866	0.001	0.942	0.616	0.466	0.648	0.066	0.668
f_Bacteroidaceae	13.0	6.0	12.8	10.5	8.8	10.7	10.4	9.3	1.997	0.427	0.138	0.570	0.763	0.489	0.081	0.171
f_Lactobacillaceae	5.9	6.7	5.5	6.5	4.5	5.2	7.8	7.4	1.118	0.127	0.493	0.939	0.768	0.065	0.638	0.666
f_Turicibacteraceae	0.53	0.73	0.51	0.34	0.85	0.31	0.58	0.63	0.181	0.486	0.362	0.609	0.654	0.369	0.315	0.063
f_Coriobacteriaceae	0.23	0.28	0.17	0.21	0.26	0.22	0.19	0.24	0.031	0.064	0.292	0.706	0.389	0.365	0.402	0.208
f_Bifidobacteriaceae	0.094	0.18	0.072	0.067	0.30	0.31	0.13	0.13	0.088	0.056	0.712	0.066	0.698	0.375	0.800	0.764
f_Peptostreptococcaceae	0.064	0.084	0.064	0.083	0.095	0.075	0.11	0.070	0.022	0.833	0.706	0.343	0.688	0.811	0.096	0.717
f_[Mogibacteriaceae]	0.049	0.037	0.042	0.033	0.039	0.042	0.037	0.038	0.005	0.176	0.219	0.716	0.953	0.759	0.071	0.725
f_Leuconostocaceae	0.009	0.058	0.037	0.017	0.031	0.032	0.033	0.035	0.012	0.811	0.363	0.762	0.057	0.597	0.449	0.042
o_RF39	0.020	0.024	0.009	0.018	0.012	0.043	0.002	0.008	0.013	0.096	0.179	0.873	0.591	0.441	0.567	0.423
Genus																
g_[Ruminococcus]	12.8	16.2	13.5	11.8	14.7	12.8	11.5	13.2	1.167	0.050	0.637	0.513	0.630	0.797	0.548	0.011
g_Bacteroides	13.0	6.0	12.8	10.5	8.8	10.7	10.4	9.3	1.997	0.427	0.138	0.570	0.763	0.489	0.081	0.171
f_Ruminococcaceae1	7.8	8.3	6.3	8.1	9.0	8.2	6.6	6.7	0.646	0.003	0.368	0.998	0.260	0.236	0.113	0.838
g_Lactobacillus	5.9	6.7	5.5	6.5	4.5	5.2	7.8	7.4	1.118	0.127	0.493	0.939	0.768	0.065	0.638	0.666
g_Oscillospira	5.2	5.1	4.6	4.9	5.0	5.0	5.0	4.1	0.317	0.054	0.390	0.458	0.644	0.844	0.204	0.135
g_Ruminococcus	3.6	3.8	3.2	3.0	3.4	3.0	3.4	3.3	0.270	0.214	0.503	0.507	0.909	0.068	0.462	0.405
g_Dorea	1.3	1.7	1.4	1.3	1.7	1.7	1.2	1.1	0.191	0.013	0.930	0.861	0.248	0.134	0.380	0.547
f_Lachnospiraceae2	0.70	0.62	0.68	0.57	0.59	0.55	0.48	0.64	0.058	0.567	0.736	0.062	0.333	0.818	0.061	0.184
g_Turicibacter	0.53	0.73	0.51	0.34	0.85	0.31	0.58	0.63	0.181	0.486	0.362	0.609	0.654	0.369	0.315	0.063
g_Coprobacillus	0.42	0.36	0.59	0.50	0.40	0.44	0.50	0.52	0.085	0.042	0.715	0.966	0.816	0.636	0.414	0.991
g_Clostridium	0.39	0.20	0.41	0.20	0.25	0.11	0.24	0.22	0.110	0.715	0.073	0.227	0.756	0.811	0.442	0.684
g_Eggerthella	0.17	0.22	0.13	0.16	0.20	0.15	0.15	0.19	0.027	0.097	0.392	0.883	0.383	0.254	0.245	0.124
g_Bifidobacterium	0.094	0.18	0.072	0.067	0.30	0.31	0.13	0.13	0.088	0.056	0.712	0.066	0.698	0.375	0.800	0.764
f_Erysipelotrichaceae1	0.12	0.19	0.16	0.049	0.17	0.12	0.070	0.098	0.049	0.143	0.648	0.654	0.429	0.837	0.854	0.065
f_Peptostreptococcaceae1	0.063	0.084	0.063	0.083	0.094	0.074	0.11	0.069	0.022	0.827	0.713	0.353	0.682	0.808	0.099	0.711
f_Ruminococcaceae2	0.097	0.090	0.068	0.091	0.086	0.065	0.079	0.052	0.011	0.131	0.311	0.042	0.444	0.825	0.044	0.260
f_[Mogibacteriaceae] 1	0.049	0.037	0.042	0.033	0.039	0.042	0.037	0.038	0.005	0.176	0.219	0.716	0.953	0.759	0.071	0.725
f_Leuconostocaceae1	0.009	0.058	0.037	0.017	0.031	0.032	0.033	0.035	0.012	0.811	0.363	0.762	0.057	0.597	0.449	0.042
o_RF391	0.020	0.024	0.009	0.018	0.012	0.043	0.002	0.008	0.013	0.096	0.179	0.873	0.591	0.441	0.567	0.423

¹CON, control diet. Values are least square means and pooled SEM; n = 10.

²Rarefaction depth of 10,000 sequences to determine α -diversity.

³Differently abundant bacterial families and genera with a relative abundance >0.01%.

Table 6. Least squares means of total and single volatile fatty acid (VFA) concentrations in cecal digesta of broiler chickens fed diets with DON and mycotoxin deactivator (B) and with or without oral LPS challenge 1 d before sampling^a

Item, µmol/g	No LPS				LPS				P-values			
	CON	CON-B	DON	DON-B	CON	CON-B	DON	DON-B	SEM	DON	B	LPS
	DON	DON-B	DON	DON-B	CON	CON-B	DON	DON-B	SEM	DON	B	LPS
Total VFA	138.4	157.1	131.1	141.8	150.8	149.1	140.0	137.7	9.67	0.106	0.352	0.738
Acetate	108.5	127.9	106.1	114.9	123.5	120.3	112.0	111.1	8.05	0.118	0.295	0.679
Propionate	6.4	5.8	5.5	5.3	6.2	6.4	4.8	5.5	0.76	0.092	0.983	0.945
Butyrate	20.7	21.0	17.3	19.0	18.4	19.7	21.3	18.9	1.62	0.474	0.834	0.973
Isobutyrate	0.97	1.25	0.84	1.04	1.08	1.32	1.03	0.99	0.35	0.463	0.492	0.746
Valerate	0.97	0.72	0.64	0.79	0.98	0.84	0.44	0.80	0.16	0.057	0.777	0.897
Isovalerate	0.79	0.38	0.59	0.64	0.56	0.51	0.37	0.52	0.10	0.626	0.342	0.116
Caproate	0.10	0.06	0.09	0.11	0.05	0.10	0.05	0.04	0.05	0.864	0.863	0.366

^aCON, control diet. Values are least square means and pooled SEM; n = 10.

Table 7. Association scores (Pearson's correlation) between the most influential OTUs and propionate and valerate concentrations in cecal digesta of chickens ($|r| > 0.40$) identified using sparse partial least squares regression and networking analysis

Item	Taxonomy	Propionate	Valerate
OTU4	Clostridiales		0.44
OTU6	Clostridiales	-0.45	
OTU61	Lachnospiraceae		0.42
OTU82	Ruminococcus		0.45
OTU85	Ruminococcaceae	0.73	0.44
OTU102	Lachnospiraceae	-0.42	
OTU110	Ruminococcus	-0.43	
OTU129	Lachnospiraceae	0.41	
OTU131	Clostridiales		0.45
OTU146	Lachnospiraceae		0.49

as one of the prime adherence targets for the commensal *E. coli* in the intestine (Al-Saedi et al., 2017). The downregulation of the MUC2 expression may be therefore a protective mechanism to lower available binding sites and substrate for the “mock *E. coli* invasion” to decrease further bacterial colonization and proliferation. As one of the major roles of IAP being the detoxification of LPS (Lallès, 2014), the trend for a lower expression of IAP at the duodenal mucosa was opposite to what we expected to observe. Since 24 h had passed, this may belong to an overshooting response after the LPS challenge and clearing of the LPS. A moderation of the mucosal response toward the two xenobiotics (i.e., DON and LPS) might be the reason for the lower duodenal expression of OCLN and TNFA compared to birds that were only exposed to DON. However, regulatory or anti-inflammatory cytokines other than the two investigated in the present study may have mediated this assumed effect, that is, TGF- β 1 and IL-10, or we missed this response. Notably, B lowered the jejunal expression of the proinflammatory NFKB and IL6 and thus proinflammatory signaling in the LPS-challenged chickens, supporting biotransformation capacities of B toward endotoxins.

Due to the absorption and metabolism of DON in the small intestine of chickens, direct effects of DON on the bacterial community may be expected in the upper segments of the gastrointestinal tract, that is, between crop and the proximal small intestine. Due to the importance of the ceca for fermentation, we focused on this segment, as any bacterial alterations, either direct or indirect, will modulate the intestine-host metabolism axis. In considering that mycotoxin exposure affects intestinal mucin glycoprotein expression and composition as well as nutrient absorption in the upper digestive tract (Pinton et al., 2015; Robert et al., 2017), changes in nutrient flow including host mucins in digesta may have led, at least in part, to the DON-associated alterations that we observed in the cecal bacterial composition. Accordingly, the majority of bacterial genera that were less abundant in chickens exposed to the dietary DON, including *Ruminococcus*, *Coriobacteriaceae*, *Bifidobacteriaceae*, *Dorea*, and *Oscillospira*, comprise strains that utilize dietary starch and fiber or degrade sugars liberated from host mucins (Gophna et al., 2017; Racheev and Thiele, 2017). *Ruminococcus*, *Coriobacteriaceae*, *Dorea*, and *Oscillospira* encompass many acetate- and butyrate-producing species, which may explain that butyrate and not propionate was more concentrated in cecal digesta in the present study. Nevertheless, in spite of the lower abundance of these predominant taxa, DON did not significantly reduce cecal acetate and butyrate concentrations. In contrast, if luminal concentrations reflect the

VFA production, DON seemed to inhibit the metabolic activity of propionate- and valerate-producing species, probably due to the aforementioned changes in nutrient flow or shifts in the availability of primary fermentation metabolites, such as lactate and succinate, for cross-feeding (Reichardt et al., 2014; Flint et al., 2015). Since we did not specifically investigate the microbial metabolism, it is difficult to specify the bacterial taxa that were responsible for the decline in propionate and valerate. Nevertheless, sPLS regression and relevance networking allowed for a tentative identification of influential taxa, showing that *Lachnospiraceae*- and *Ruminococcaceae*-OTUs mostly influenced cecal propionate and valerate concentrations. Interestingly, B alleviated the DON effects on the cecal valerate concentration. Although interactions of the cecal microbiota with bentonite and bacterial strains as components of B may be thinkable, this needs further research. The finding of a trend of lower abundance of *Clostridium* with B may be beneficial for mucosal protection and health as this genus comprises mucolytic species and some important pathobionts (e.g. *Clostridium perfringens*). Following this line of reasoning, the effects of B and LPS on the bacterial community may have been mainly indirect effects, like for DON, caused by gut physiological alterations including cecal flow of dietary residuals and mucus production.

In conclusion, despite the fact that the present contamination level surpassed the European guidance value for poultry feed, dietary DON did not affect growth performance of the present chickens; however, its cytotoxicity probably impaired the development of important immune organs (i.e., bursa fabricii and thymus) postabsorption. The addition of B modified the mucosal expression of genes related to the innate immune response in the small intestine and jejunal glucose absorptive tissue response; partly with the effect of the B depending on whether DON was present in the diet or not. The changes in the cecal microbiota composition and VFA profile were likely associated with alterations in host physiology in the small intestine caused by DON, B, and LPS.

Supplementary Data

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

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