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Effects and biotransformation of the mycotoxin deoxynivalenol in growing pigs fed naturally-contaminated grain pelleted with and without the addition of *Coriobacteriaceum* DSM 11798

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Abstract

Deoxynivalenol (DON) is one of the most prevalent *Fusarium* mycotoxins in grain and can cause economic losses in pig farming due to reduced feed consumption and lower weight gains. Biodetoxification of mycotoxins using bacterial strains has been a focus of research for many years. However, only a few *in vivo* studies have been conducted on the effectiveness of microbial detoxification of fusariotoxins. This study was therefore aimed at investigating the effect of a feed additive containing the bacterial strain *Coriobacteriaceum* DSM 11798 (the active ingredient in *Biomin®* BBSH 797) on growth performance and blood parameters, as well as uptake and metabolism of DON, in growing pigs. Forty-eight crossbred (Landrace-Yorkshire/Duroc-Duroc) weaning pigs were fed pelleted feed made from naturally-contaminated oats, with DON at four concentration levels: (1) control diet (DON < 0.2 mg kg^{-1}), (2) low-contaminated diet (DON = 0.92 mg kg^{-1}), (3) medium-contaminated diet (DON = 2.2 mg kg^{-1}), and (4) high-contaminated diet (DON = 5.0 mg kg^{-1}) and equivalent diets containing DSM 11798 as feed additive. During the first 7 days of

exposure, pigs in the highest-dose group showed a 20-28 % reduction in feed intake and a 24-34 % reduction in weight gain compared with pigs in the control and low-dose groups. These differences were levelled out by study completion. Towards the end of the experiment, dose-dependent reductions in serum albumin, globulin and total serum protein were noted in the groups fed DON-contaminated feed compared with the controls. The addition of DSM 11798 had no effect on the DON-related clinical effects or on the plasma concentrations of DON. The ineffectiveness of the feed additive in the present study could be a consequence of its use in pelleted feed, which might have hindered its rapid release, accessibility or de-toxification efficiency in the pig gastrointestinal tract.

Keywords: BBSH 797, deoxynivalenol (DON), microbial detoxification, blood parameters, biotransformation, feed additive, pigs

Introduction

Deoxynivalenol (DON), also known as vomitoxin, is produced by fungal pathogens belonging to the genus *Fusarium* and, in particular, *F. graminearum* and *F. culmorum*. DON can occur in oats, wheat, barley and corn and is the most commonly detected trichothecene mycotoxin in cereal grains in Northern countries (Vitenskapskomiteen for mattrygghet (VKM) 2013). Pigs are more sensitive to DON exposure than other farm animals, possibly because of differences in uptake (Bracarense et al. 2012). Acute exposure of pigs to high doses of DON can cause vomiting (Forsyth et al. 1977). Consumption of feed that is naturally contaminated with moderate levels of DON has been shown to induce dose-related feed refusal, unrest, decreased feed intake and decreased weight gain. Pigs fed with diets containing 1.7 and 3.5 mg DON kg^{-1} have shown partial feed refusal (Bergsjo et al. 1993), whereas 40 mg DON kg^{-1} has caused complete feed refusal (Forsyth et al. 1977). The reported Lowest Observed Adverse Effect Levels (LOAEL), which is based on reduced feed intake, has varied from 0.35 to 2 mg DON kg^{-1} in feed in different studies (Vitenskapskomiteen for mattrygghet (VKM) 2013). Toxic effects may impair swine health and welfare, production results, and lead to economic losses (Wu 2007).

DON and its fungal derivatives 3-*O*-acetyl-DON and 15-*O*-acetyl-DON, as well as the plant detoxification product DON-3-*O*-ß-D-glucoside, become bioaccessible in pigs after oral uptake either directly or after hydrolytic cleavage. Water-soluble DON is rapidly absorbed and reaches the systemic circulation in less than 15 min (Broekaert et al. 2017; Goyarts and Danicke 2006). The bioavailability of DON from ingested feed in pigs is high, with estimates between 50 and 90 %. DON is metabolised in pigs to the main conjugated products DON-3-*O*-ß-D-glucuronide and DON-15-*O*-ß-D-glucuronide, primarily by hepatic enzymes. Iso-DON-glucuronides and other metabolites are also detected in rodents, ruminants and pigs, but as minor metabolites (Schwartz-Zimmermann et al. 2017). The plasma half-life of the mycotoxin is in the range of 3 to 5 h. Excretion in pigs is relatively slow compared with that in other species, resulting in considerable exposure, which is regarded as one reason for the relatively high sensitivity of pigs to DON (Goyarts and Danicke 2006).

The risk to animal health from the exposure to DON has been assessed by the European Food Safety Authority (EFSA 2004). Considering the particular sensitivity of pigs, the recommended maximum acceptable level for DON, according to European Commission Recommendation 2006/576/EC, is set to 0.9 mg kg^{-1}. In Norway, the national feed safety authority recommends an even lower level of 0.5 mg DON kg^{-1} for pig feed (Mattilsynet 2015), causing feed manufacturers and farmers additional efforts to provide suitable feed grain sources. Thus, detoxifying measures allowing the utilisation of contaminated grain lots are of great interest.

Strategies to reduce exposure to mycotoxins include the supplementation of feed products with detoxifying additives, such as passive mycotoxin adsorbents, chemical supplements or active biotransforming agents containing bacteria, fungi or enzymes that can degrade mycotoxins into non-toxic metabolites (Jard et al. 2011). Adsorbing materials are less effective in binding trichothecene mycotoxins, such as DON (Awad et al. 2010). Therefore, biological detoxification methods targeting trichothecenes have been a research focus in recent years. Ruminal microbiota biotransform DON efficiently to products of less or no toxicity and are responsible for the relative tolerance of ruminants towards increased levels in feed (Seeling and Dänicke 2005). The safety and efficacy of the ruminal microbe *Coriobacteriaceae gen. nov. sp. nov.* DSM 11798, provided as the active ingredient of Biomin® BBSH 797 by Biomin (Herzogenburg, Austria), has been assessed by EFSA for use in pigs (European Food Safety Authority (EFSA) 2005; 2013) and has been approved by the European Commission (European Union 2013). Based on the available documentation, a minimum content of 1.7×10^8 colony-forming units (cfu) kg^{-1} feed (with 12 % moisture) was recommended to achieve sufficient detoxification (European Food Safety Authority (EFSA) 2013).

DSM 11798 detoxifies DON by the reduction of the 12,13-epoxy group to produce de-epoxy-DON (DOM-1). The de-epoxidation activity has been shown *in vitro* under anaerobic conditions and *ex vivo* in simulated swine gut (Fuchs et al. 2000; Fuchs et al. 2002; Schatzmayr et al. 2006). *In vivo* efficacy of DSM 11798 in weaned pigs fed with DON-spiked or naturally contaminated non-pelleted feed has been reported in short-term studies, showing significant clinical effect on performance (Cheng et al. 2006; Plank et al. 2009) and an effective decrease of DON serum concentrations (European Food Safety Authority (EFSA) 2013; Starkl et al. 2015), whereas the results were ambiguous in other performance studies with fattening pigs (European Food Safety Authority (EFSA) 2005). Another study, adding the clay-matrix stabilised bacterium (BBSH 797) as a ready-made product (Mycofix® Plus) to feed containing highly contaminated maize (3.5 - 8 mg DON kg^{-1} feed), in an experiment with fattening pigs, did not demonstrate significant performance improvement by the detoxifying supplement (Preißinger et al. 2016).

The aim of the present study was to elucidate the effects of chronic exposure to low, moderate and high levels of DON in pelleted feed produced from naturally contaminated oats with and without DSM 11798 addition by analysing feed uptake, growth performance, clinical parameters and plasma levels of DON and its metabolites in growing pigs.

Materials and methods

Animals and housing

In a 42-day experiment, 48 crossbred (Landrace-Yorkshire/Duroc-Duroc) five-week-old weanling pigs

of both sexes (24 castrated and 24 females) with a mean initial weight of 11.0 ± 1.5 kg were individually housed in floor pens with openings for social contact in environmentally controlled rooms. The experiment was run in two rounds with identical procedures including 24 pigs in each round of experiment. The first round was conducted in July and August 2014, and the second round, in September and October 2014. The temperature in the animal facility was at 25 °C during the first round, and 23 °C, during the second. At the beginning of each round, pigs were divided into 8 feeding groups of six animals, with equal numbers of females and castrated males within the groups. The pigs received routine anthelmintic treatment (Panacur vet. (fenbendazol), MSD Animal Health) and were allowed seven days acclimatisation to the environment and diet before the study was initiated. In this period, all pigs received the control diet. Throughout the experiment, both water and feed were provided *ad libitum* using automatic feeders (DOMINO-Feeder: Slop Feeder K-1 (08261) and drinking cups (DOMINO-Drinking cups: H2O (49014), Domino A/S, Tørring, Denmark). The automatic feeders were inspected daily, and the settings were adjusted individually to minimize feed loss. At study completion, the pigs were euthanized by captive bolt pistol. The animals were bled out, and the organs of interest dissected for further analysis. The Norwegian Animal Research Authority (Groenen et al.) approved the study and all experimental procedures (Approval no. 6707 – 2014).

Origin of the naturally contaminated oats

The oats used for the production of the experimental diets in the present study had been harvested in southern Sweden in 2013. Because of its high content of deoxynivalenol (DON), this specific oat batch was excluded from use in feed or food and was bought for use in the pig study.

Preparation of the experimental diets

After the 1-week (day -6 to day 0) acclimatisation, each group received pelleted feed containing different levels of naturally DON-contaminated oats (Table 1). The following DON levels were achieved in the feed: (1) control diet (DON < 0.2 mg kg^{-1}), (2) control diet supplemented with a feed additive containing the trichothecene-degrading bacteria DSM 11798, (3) low-contaminated diet (DON = 0.92 mg kg^{-1}), (4) low-contaminated diet (1.0 mg kg^{-1}) with DSM 11798, (5) medium-contaminated diet (DON = 2.2 mg kg^{-1}), (6) medium-contaminated diet (2.5 mg kg^{-1}) with DSM 11798, (7) high-contaminated diet (DON = 5.0 mg kg^{-1}) and (8) high-contaminated diet (5.7 mg kg^{-1}) with DSM 11798 (Table 1). The feed additive containing *Coriobacteriaceae Gen. nov. sp. nov.* DSM 11798 in a non-commercial form was supplied by Biomin®, Herzogenburg, Austria, as a dry grey-brown powder containing less than 35 % cell mass and 40 – 60 % coating agent. The different levels of DON were achieved by blending the naturally contaminated oats at three ratios with very low-contaminated oats harvested in southern Norway in 2013. The pelleted diets (Table 1) were formulated at Felleskjøpet Fôrutvikling (Trondheim, Norway) and produced at the Centre for Feed Technology at the Norwegian University of Life Sciences (Fôrtek, Ås, Norway) by a standard pelleting process, keeping the pelleting temperature close to 80 °C. The diets were produced immediately before the first round of the experiment and were stored dry at room temperature until the second round.

Analysis of number of active DSM 11798 bacteria in experimental diets

The numbers of cfu of DSM 11798-containing was measured at the Biomin Research Centre (Tulln, Austria) in the finished feed additive and in the different diets after pelleting using a validated enumeration method based on Koch's pour plate method: bacteria were extracted from the feed additive and the milled diets and serially diluted in a suitable medium under anaerobic conditions. Aliquots (1 ml) of three dilutions were transferred to Petri dishes and mixed with supplemented agar medium. After incubation under strictly anaerobic conditions at 37 °C in an incubator with 100 % CO_2 atmosphere, bacterial colonies were counted by transient light microscopy, and the number of DSM 11798 cfu g^{-1} in the different samples was calculated (Table 1). The test was performed in triplicate.

Reagents for chemical analyses

Acetonitrile, methanol and water (Fisher Scientific, Fair Lawn, NJ, USA) for liquid chromatography high-resolution mass spectrometry (LC–HRMS) analysis were of Optima™ LC–MS quality, while acetonitrile for sample preparation was from Romil (Cambridge, UK). Ammonium acetate and glacial acetic acid were of p.a. quality (Merck, Darmstadt, Germany). 4-Deoxynivalenol (DON), deepoxy-DON (DOM-1), 3-*O*-acetyl-DON (3-ac-DON) and DON-3-*O*-ß-D-glucoside (DON-3-Glc) were purchased from Romer Labs (Tulln, Austria), while DON-3-*O*-ß-D-glucuronide (DON-3-GlcA) and DON-15-*O*-ß-D-glucuronide (DON-15-GlcA) were available at the NVI, as described in a previous work (Uhlig et al. 2013).

Sample preparation of experimental diets for analysis of mycotoxins

Samples of 2.5 g from each of the experimental diets were milled with a Retsch ZM 100 mill (Retsch GmbH & Co. KG, Haan, Germany) and placed into 50-ml centrifuge tubes, and 10 ml of MeCN/H_2O/formic acid (80:19.9:0.1, v/v/v) was added. The mixture was vortexed for 30 s and extracted for 30 min using a horizontal shaker at 175 min^{-1}. After centrifugation at 4.000 × g for 10 min (4 °C), the supernatants were transferred into clean 50-ml tubes, and the remaining solid material was extracted with 10 ml of MeCN/H_2O/formic acid (20:79.9:0.1, v/v/v) by shaking for an additional 30 min. The two extracts were combined and kept at 4 °C before final centrifugation (4.000 × g, 10 min and 4 °C). Finally, a 0.5-ml aliquot of the combined supernatants was centrifuged for 1 min at 15.000 × g through 0.22-µm nylon filters (Costar Spin-X 0.22 Nylon filter; Corning, Inc., Corning, NY, USA) and analysed using liquid chromatography high-resolution mass spectrometry (LC-HRMS).

Analysis of mycotoxins in experimental diets

The samples of the experimental diets were analysed for DON, DON-3-Glc, 3-ac-DON and 15-ac-DON with a previously validated LC-HRMS method (Ivanova et al. 2017). The limits of detection (LOD) in a feed matrix were 14 µg kg^{-1} for DON, 26 µg kg^{-1} for DON-3-Glc, 5.9 µg kg^{-1} for 3-ac-DON and 52 µg kg^{-1} for 15-ac-DON. In addition, the eight experimental diets were analysed for a range of other mycotoxins using a semi-quantitative multi-toxin screening method at the Centre for Analytical Chemistry at IFA Tulln, Austria, using a semi-quantitative multi-toxin screening method (Malachova

et al. 2014).

Recording of feed intake and growth performance

Individual body weight (BW) was measured twice during the acclimatisation and thereafter twice weekly until the last day of the experiment using a digital platform scale (KRUUSE, PS250, Langeskov, Denmark). Feed consumption was also measured individually by weighing each of the automated feeding stations using the same platform scale every time new feed was added, and additionally three times a week throughout the study. Average Daily Feed Intake (ADFI), Average Daily Weight Gain (ADG) and Feed Conversion Ratio (FCR) were calculated based on the registration of feed consumption.

Clinical chemistry and haematology

All blood samples were collected from the jugular vein using 5-ml Vacutainer tubes. Blood samples for clinical evaluations were collected on day -3 (week 0) in the acclimatisation period and thereafter on d 4 (week 1), d 18 (week 3), and d 35 (week 5) during the period with DON exposure. Blood for haematology was collected in 5-ml tubes, and EDTA was used as an anticoagulant. The sampled blood was kept refrigerated and delivered to the Central Laboratory of the NMBU-Faculty of Veterinary Medicine (Oslo, Norway) for analysis on the same day. Blood for serum biochemistry was collected in 5-ml tubes with gel and clot activating factor. The tubes were kept at room temperature for 1–2 h. Serum was then separated by centrifugation at 1500 × g for 10 min at room temperature, stored frozen in 2-ml cryogenic vials (Nalgene, Nalge Company, Rochester, NY, USA), and delivered to the Central Laboratory.

Haematologic analyses (CBCs) were performed upon arrival in the laboratory using ADVIA® 2120 Haematology System and ADVIA® Multispecies software (Siemens Healthcare Diagnostics, Siemens AG, Germany) using the settings for swine. The clinical biochemical analyses were performed using ADVIA 1800® Clinical Chemistry System (Siemens Healthcare Diagnostics, Siemens AG, Germany), and serum protein electrophoresis was performed on a Sebia CapillarysTM 2 (Sebia, Norcross, GA, USA).

Sample preparation of plasma for analysis of mycotoxins

Blood samples for measuring plasma concentrations of DON, DOM-1, DON-3-GlcA and DON-15-GlcA were collected once on day -3 in the acclimatisation week and thereafter three times a day (08:00, 11:00 and 16:00) on days 1, 4, 11, 18, 25, 32 and 35 during the period with DON exposure. Blood for analysis of DON and its metabolites was collected in 3-ml lithium-heparin Vacutainer tubes. Plasma was separated by centrifugation at 1500 × g for 10 min at room temperature (approximately 20 ℃) and stored frozen in 2-ml cryogenic vials (Nalgene, Rochester, NY, USA). Plasma samples (250 µL) were transferred into conical 15-ml plastic tubes (Corning Inc., Corning, NY, USA), mixed with 750 µL of acetonitrile, vortexed for 15 s, and sonicated (Branson 3200, Emerson, St. Louis, MO, USA) for 5 min. Proteins were precipitated by centrifugation at 2000 × g for 10 min at 4°C (Beckman Coulter, Brea, CA, USA), and supernatants were transferred to 10-ml conical glass tubes and evaporated to

dryness at 60°C using a gentle stream of pure nitrogen, quality 6.0. Dried samples were stored refrigerated, dissolved in 200 µL of water, vortexed for 15 s, sonicated for 5 min and transferred to HPLC vials prior to LC-HRMS analysis.

Analysis of mycotoxins in pig plasma

Plasma was analysed for DON, DOM-1, DON-3-GlcA and DON-15-GlcA. Samples were chromatographically separated at 30 °C using a UHPLC Dionex Ultimate 3000 system (Thermo Fisher Scientific, Waltham, MA, USA) with a 100 × 2.1 mm i.d. Acquity UPLC HSS T3 column (1.8 µm; Waters, Milford, MA, USA) and a 5 × 2.1 mm i.d. XSelect HSS T3 VanGuard pre-column (2.5 µm, 100 Å, Waters). The flow rate of the mobile phase was 0.5 ml min^{-1}, and the injection volume was 6 µL. Eluent A was water, and eluent B was 95% acetonitrile (both containing 5 mM ammonium acetate and 0.1% acetic acid). The column was eluted isocratically with 100 % A for 1 min and then, using a linear gradient, to 15 % B in 15 min. After flushing the column for 2.5 min with 100 % B, the mobile phase composition was returned to the initial conditions, and the column was eluted isocratically for 2.9 min. The LC-system was on-line coupled to a Q-Exactive™ Hybrid Quadrupole-Orbitrap high-resolution mass spectrometer (Thermo Fisher Scientific) equipped with a heated electrospray ion source (HESI-II). The HESI-II interface was operated at 300 °C in the negative ionisation mode, and the parameters were adjusted as follows: spray voltage, 4 kV; capillary temperature, 250 °C; sheath gas flow rate, 35 L min^{-1}; auxiliary gas flow rate, 10 L min^{-1}; and S-lens RF level, 55. The data were acquired in the selected ion monitoring (Boudergue et al.)/data-dependent MS2 (dd-MS2) mode targeting the $[M+acetate]^-$ ions for DON and DOM-1 (m/z 355.1387 and 339.1438, respectively) and the $[M-H]^-$ ions for the DON-glucuronides (m/z 471.1497) with a quadrupole isolation width of 2 *m/z* and a mass resolution of 70.000 full width half-maximum (FWHM) at *m/z* 200 for SIM. The presence of a target ion above a threshold intensity of 5×10^3 triggered a MS^2 scan for analyte verification (dd-MS2) using a normalised collision energy of 35%. The mass resolution during dd-MS2 was set to 17.500 FWHM. The automatic gain control (AGC) target was set to 5×10^5 ions including a maximum injection time (IT) of 250 ms during SIM, whereas for dd-MS2 the AGC target was 5×10^4, and the IT was 200 ms.

Matrix-matched, 1/x weighed calibration curves were plotted for DON, DOM-1, DON-3-GlcA and DON-15-GlcA using blank pig plasma. Xcalibur version 2.2 or 2.3 (Thermo Fisher Scientific) was used for data processing. Each round of analyses included at least one blank pig plasma sample, which had been fortified with 5.3 or 26 ng ml^{-1} of DON, DON-3-GlcA and DON-15-GlcA and 11 or 52 ng ml^{-1} DOM-1. The overall spike recoveries were (standard deviations in parentheses) DON 80 (11) %, DOM-1 74 (12) %, DON-3-GlcA 67 (17) % and DON-15-GlcA 65 (14) %. The limits of detection (LOD) were 0.1 ng ml^{-1} for DON, 0.2 ng ml^{-1} for DOM-1, 1.5 ng ml^{-1} for DON-3-GlcA and DON-15-GlcA.

Statistical analyses

Performance and haematological and biochemical data were analysed by repeated-measures analysis using a mixed model in JMP®, Version 10 (SAS Institute Inc., Cary, NC, USA). The level of significance was set to 0.05 in all models, and results with *p*-values between 0.05 and 0.1 were considered significant trends. If not otherwise specified, all results are expressed as the mean ± standard deviation (SD). The data were considered as a completely randomized block design with

eight treatments in six blocks. Each pig was considered as random effect and represented an experimental unit for the variables tested. The DON-levels and feed additive (DSM 11798) were considered independent variables. ADFI, ADG, FCR, haematological and biochemical parameters, and plasma concentrations of DON and its metabolites were defined as dependent variables. Performance and blood parameters were subjected to repeated-measure ANOVA according to a three-factorial design:

$Y_{ijkl} = Л + a_i + b_j + c_k + a.b_{(ij)} + a.c_{(ik)} + b.c_{(jk)} + a.b.c_{(ijk)} + e_{ijkl,}$ *where* Y_{ijkl} is the *lth* observation related to the DON-levels *i,* feed additive (with or without DSM 11798) *j*, and time of exposure *k*; *Л* is the overall mean; a_i is the effect of DON-levels; b_j is the effect of feed additive (DSM 11798); c_k is the effect of time; $a.b_{(ij)}$ is the interaction between DON-levels and feed additive, $a.c_{(ik)}$ is the interaction between DON-levels and time; $b.c_{(jk)}$ is the interaction between feed additive and time; $a.b.c_{(ijk)}$ is the interaction between DON-levels, feed additive and time; and e_{ijkl} is the error term.

The normality of distribution of the different parameters was controlled by residual and predicted values plot, normal-percentile plots and Shapiro-Wilk test. If the *p*-value in the Shapiro-Wilk test was over 0.05, the data were considered normally distributed. The data that were not normally distributed were transformed or analysed by non-parametric models, such as Wilcoxon´s rank sum test. Levene's test was used to check the assumption of homogeneity of variances. If the *p*-value of Levene's test was over 0.05, variances were considered equal. If the output generated from the application of repeated-measure ANOVA was significantly different, the post hoc Tukey-Kramer HSD test was used for multiple comparisons and the identification of significant differences ($p < 0.05$).

Plasma concentrations of DON and its metabolites were evaluated by the non-parametric Wilcoxon Each Pair test ($p < 0.05$). In the statistical calculations, concentrations below the limit of detection (LOD) were represented by the LOD divided by the square root of 2.

Results

Effects of experimental diets on feed intake and growth performance.

The piglets accepted the oat-based feed (64 % oat; Table 1) easily, showed no signs of digestive problems, interacted socially with their neighbours, and became accustomed to being handled and weighed. The animals did not show obvious signs of toxicity, such as vomiting, disease or distress related to the experimental diets, at any time during the 42-day study.

Table 2 summarises the data on the effects of DON-contaminated diets with and without DSM 11798 on feed intake and growth performance during the experimental period. The data generated from the application of repeated-measure analysis of variance (ANOVA) on the growth performance parameters and related interactions between different effects are shown in Table 2b. The results from day 15 to 21 (week 3) and day 22 to 28 (week 4) of the exposure period (Table S2) were intermediate between results from week 2 and week 5 and are omitted from Table 2a to reduce the size of the table. The average daily feed intake (ADFI), average daily weight gain (ADG) and feed conversion ratio (FCR) were similar among all treatments during the pre-exposure period. The six-week old pigs ($n = 48$) had a mean ADFI of 337 ± 86 g day^{-1} and a mean ADG of 243 ± 85 g

day^{-1}, resulting in a mean FCR of 1.47 ± 0.37 and a total weight gain of 1.71 ± 0.59 kg in the acclimatisation week (week 0; day -6 to day 0) by consumption of the oat control diet.

When evaluated over the entire experimental period, neither ADFI nor ADG were significantly influenced by the exposure to DON or the presence of DSM 11798 in the experimental diets. However, on week 1 (day 1 to 7) of the exposure period, pigs in the high-DON group showed a significant ($p < 0.05$) 24% and 34% reduction in ADG compared with those fed with the control and low-DON groups, respectively. This trend was also observed, although the difference was not significant, in the medium-DON group (20 % ADG reduction in relation to the low-DON group). The addition of DSM 11798 did not show any effect on the ADFI, ADG or FCR results. On week 2 (day 8-14) of the DON exposure period, differences in ADG between the high-DON group and the control and low-DON groups were still noticeable, but the gap had decreased and was no longer significant. In subsequent weeks, the pigs appeared to adapt to the contaminated diets so that at the end of the feeding experiment (week 5), both ADFI and ADG were normalized and comparable between all DON-exposed and control groups. The mean total weight gain in the DON exposure period (days 1-35) in all diet groups was 24.7 ± 3.6 kg, the mean feed intake was 35.6 ± 6.1 kg, and the mean FCR was 1.57 ± 0.08 ($n = 48$).

Effects of experimental diets on haematological and biochemical parameters

Biochemical parameters

No differences in any of the measured biochemical parameters were observed between the two groups at the same dose level receiving feed with and without the addition of DSM 11798 throughout the experiment. Comparisons between DON dose levels are therefore based on $n = 12$ per group. Figure 1 shows the effects of experimental diets on some selected serum biochemical parameters of pigs in the experimental period. The studied serum biochemical parameters did not differ between the treatment groups on week 0 and week 1 (Figure 1). However, total serum protein concentrations were significantly ($p < 0.05$) lower in pigs in the high-DON group than in those in the control group in week 3 (Figure 1a). This difference was further increased in week 5, where pigs in the high-DON group had significantly lower total serum protein concentrations than pigs in the control ($p < 0.0001$) and low-DON groups ($p < 0.05$). In addition, pigs in the medium-DON group had significantly ($p < 0.05$) lower total serum protein concentrations than in pigs in the control group.

Serum albumin concentrations (Figure 1b) and total serum globulin concentrations (Figure 1c) showed a similar dependency. The pigs in the high-DON group had significantly ($p < 0.05$) lower serum albumin than pigs in the control group on week 5. Total serum globulin was significantly ($p < 0.05$) lower in the high-DON group than in the control group in week 3 and 5. These findings support the assumption that the observed effects were dose-dependent. The effects in the low-DON and medium-DON groups were also noticeably though not significantly different from those in the control group (Figure 1a, b and c).

The analysis of serum calcium concentrations showed that pigs in the high-DON group had significantly ($p < 0.05$) lower levels of calcium than pigs in the control group on week 5 (Figure 1d). Other serum biochemical parameters, such as aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transpeptidase, glutamate dehydrogenase, creatine kinase, C-reactive protein,

urine acid, creatinine, total bilirubin, cholesterol, glucose, inorganic phosphate, magnesium, sodium, potassium and iron, were not significantly affected by different DON levels in the feed.

Haematological parameters

Haematology parameters (CBS factors), including red and white blood cell counts, haematocrit, haemoglobin and platelets, did not change observably with dose scheme, time or addition of DSM 11798.

DON and its metabolites in pig blood

The plasma concentrations of DON and its main metabolites in pig, such as DOM-1, DON-3-GlcA and DON-15-GlcA, were determined in all pigs in all dose groups once in the acclimatisation period and at eight sampling days during the five weeks of DON exposure. On each sampling day, three samples were taken in the morning, around noon and in the afternoon, with the aim to mirror the feeding rhythm of the pigs. The plasma analysis showed, however, that the concentrations of DON and its metabolites were relatively constant throughout the day, likely due to the *ad libitum* access to feed. Therefore, the three measured values per animal on each sampling day were combined to obtain average values, with which a mean for each dose group (n = 6) was calculated.

Figure 2 presents the measured plasma concentrations of DON, DOM-1, DON-3-GlcA and DON-15-GlcA during the study. Whereas the average levels were low in the control pigs (0.6 ± 0.2 ng ml^{-1}), DON and DON-metabolites were detected in a dose-dependent pattern in the pigs fed with low-, medium- and high-DON contaminated diets.

The pairwise comparison between groups receiving the same dose, with or without the addition of DSM 11798, showed that the levels of DON (Figure 2a) and DOM-1 (Figure 2b) were very similar. In contrast, the glucuronide metabolites, DON-3-GlcA (Figure 2c) and DON-15-GlcA (Figure 2d), were elevated in samples from pigs in the low-DON, medium-DON and high-DON groups receiving DSM 11798-supplemented feed. The effect was significant for DON-3-GlcA on day 4 in high-DON and medium-DON, on day 11 in high-DON, medium-DON and low-DON, on day 18 in high-DON and medium-DON, on day 25 in medium-DON and low-DON, on day 32 in medium-DON and on day 35 in low-DON ($p < 0.05$). The effect was significant for DON-15-GlcA in medium-DON on days 4 and 11 ($p < 0.05$). No sex-dependent differences were found in the plasma levels; neither of DON, DOM-1 nor of the DON-glucuronides.

Considering the mean feed intake and pig weights including gain, the approximate average DON doses (µg kg^{-1} body weight day^{-1}) in the toxin-exposed groups were calculated for each week of the study period (Table 3). The estimated doses were almost constant throughout the study due to a practically parallel increase in body weight and feed intake. This resulted in nearly stable plasma concentrations in the pigs at each DON level during the experiment. Calculated for the whole exposure period, the mean DON plasma concentrations were 3.5 ± 1.6 ng ml^{-1}, 9.4 ± 3.9 ng ml^{-1} and 16.0 ± 6.0 ng ml^{-1} in pigs receiving low, medium and high-DON diets, respectively. The average DOM-1 plasma concentrations were found to be 0.4 ± 0.3 ng ml^{-1}, 0.9 ± 0.5 ng ml^{-1} and 1.6 ± 0.8 ng ml^{-1} in pigs fed low-, medium- and high-DON diets, respectively. The average DON-3-GlcA concentrations in plasma were 3.1 ± 1.8 ng ml^{-1}, 11.1 ± 5.7 ng ml^{-1} and 19.0 ± 9.0 ng ml^{-1}, and the average DON-15-GlcA

plasma concentrations were 3.0 ± 1.6 ng ml^{-1}, 8.1 ± 4.3 ng ml^{-1} and 15.0 ± 7.4 ng ml^{-1} in pigs fed with low-, medium- and high-DON contaminated diets, respectively.

Discussion

In this study, reductions in feed intake and reduced weight gain in the first week of the exposure period were the major adverse clinical effects of feeding animals with DON-contaminated diets. In this first week, feeding of high-contaminated diets led to 20-28 % reduction in feed intake and 24-34 % reduction in weight gain compared with the control and low-contaminated diet groups. However, these variables were normalised in the subsequent weeks. No significant differences in feed intake and daily weight gains were observed between groups in weeks 2, 3, 4 and 5 or over the entire 35-day exposure period.

An equivalent, transient effect during the first week of DON exposure has been reported previously in pigs fed diets naturally contaminated with DON (2.8 mg kg^{-1}) (Wache et al. 2009). Similar observations of feed aversion and particular effects on growth performance in the first weeks of feeding with DON-contaminated feed have also been reported by other authors (Bergsjo et al. 1993; Wache et al. 2009). Several mechanisms have been proposed to contribute to the reduction in feed intake induced by DON-contaminated feed. Some studies have shown that DON can cross the blood-brain barrier, leading to activation of central structures and affecting glial cell viability and function (Behrens et al. 2015; Razafimanjato et al. 2011). Other experiments have suggested a DON-induced reduction in plasma insulin-like growth factor acid-labile subunit (IGFALS) (Flannery et al. 2013) and an increased pro-inflammatory cytokine expression (Pestka and Amuzie 2008), effects that initiate anorexia and poor growth performance. In addition, it has been reported that acute ip-exposure of mice to DON (1-5 mg kg^{-1}) may elevate levels of the gut satiety hormones peptide YY (PYY) and cholecystokinin (CCK) and that this may be related to DON-induced feed refusal and growth suppression (Flannery et al. 2012).

Another possible explanation is that DON as a physiological or systemic stressor activates the hypothalamus-pituitary-adrenal (HPA) axis and can induce a corticosterone stress response, an effect that has been documented both in broiler chickens and in mice (Antonissen et al. 2016; Islam and Pestka 2003). A stress situation can in itself lead to reduced feed intake and poor growth performance in pigs (Campbell et al. 2013). The observed transience of the first-week effects of DON on feed intake could be in agreement with proposals in a review by Grissom and Bhatnagar (2009), suggesting possible habituation of HPA responses to repeated exposures to stressors.

Calculated over the entire 35-day exposure period, the effect of DON-contaminated feed on ADG was not significant. However, in previously reported studies with DON concentrations at similar levels, the effect over medium-length periods of exposure has also been variable. While Plank et al. (2009)showed a significant effect of 2 mg DON kg^{-1} feed in approximately 6 weeks of exposure and Bergsjø et al. (1993) showed significant effect of 3.5 mg DON kg^{-1} feed in 12 weeks of exposure. Bergsjø et al. (1992) in another study found statistically significant effect of 4 mg DON kg^{-1} on performance in the first 8 weeks of experiment. Øvernes et al. (1997) did not find significant effect of 4.7 mg kg^{-1} on performance during the first 8 weeks of exposure when the pigs were fed *ad libitum.*

The results of the present study also indicate that feeding pigs with DON-contaminated diets can contribute to significant changes in serum protein levels. In our experiment, total serum protein,

globulin and albumin were lower in pigs fed contaminated diets after 3 weeks of exposure and at the end of the feeding experiment (Figure 1). This finding is in agreement with other studies, which showed significant reductions in total serum protein and/or globulin in pigs fed diets contaminated with DON (3 to 4 mg kg^{-1}) (Bergsjo et al. 1993; Rotter et al. 1995). However, there are also studies that have not shown any significant effect of dietary exposure to DON on blood protein concentrations in pigs (Dänicke et al. 2004; Goyarts et al. 2006; Wu et al. 2015). It has been proposed that the reduction in serum protein levels observed in a proportion of feeding experiments may be a result of impaired protein synthesis in the liver related to reduced feed uptake when pigs are fed DON-contaminated diets (Rotter et al. 1995). In the present study, however, the effect on serum protein levels was evident in the last weeks of the experiment, when feed intake and weight gain were not significantly affected. This finding may indicate that the reduction in serum protein levels was an independent, direct effect of DON.

A significant inverse relationship with serum calcium and a trend ($p < 0.1$) of reduced serum phosphorous concentrations in pigs fed the high-DON feed (5.3 mg kg^{-1}) was another significant change in the last week of the present study. As these effects were only seen in week 5, and there was no tendency in the same direction in previous weeks, we cannot exclude that the result is accidental. However, if the finding reflects a real effect, it is in line with results from some other experiments (Bergsjo et al. 1993; Prelusky et al. 1994; Rotter et al. 1995). The possible mechanism behind the effect is unknown, but it might be related to changes in intestinal absorption related to DON-induced changes in intestinal morphology, permeability and transporter functions (Alizadeh et al. 2015; Maresca et al. 2002).

The *Coriobacteriaceae* strain DSM 11798, also known as BBSH 797, is a Gram positive, strictly anaerobic strain originally isolated from bovine rumen (European Food Safety Authority (EFSA) 2013). Several *in vitro* studies have confirmed that this bacterial strain can reduce DON to the less toxic metabolite DOM-1, also under simulated gut conditions including low pH values (Fuchs et al. 2000; Fuchs et al. 2002; Schatzmayr et al. 2006). Rather few *in vivo* studies in pigs have investigated the efficacy of the stabilised DSM 11798 strain (European Food Safety Authority (EFSA) 2005; 2013; Plank et al. 2009). Other studies made use of commercial, mixed feed additives, which contained a combination of DSM 11798 (as BBSH 797), patented specific enzymes and other components (Cheng et al. 2006; Grenier et al. 2013).

In this present study, DON-contaminated diets impaired performance parameters in the pigs to a moderate degree and addition of DSM 11798 to experimental diets had no influence on the growth performance. This finding is in agreement with some previous studies, where DSM 11798 did not show any effect on performance parameters (European Food Safety Authority (EFSA) 2005; Preißinger et al. 2016). However, our results are inconsistent with those of other previous studies, which have indicated a positive effect of DSM 11798/BBSH 797 on the performance of pigs fed DON-contaminated grain (European Food Safety Authority (EFSA) 2005; Plank et al. 2009).

In the current study, changes in serum chemical parameters in pigs fed DON-contaminated diets were not improved by feed supplementation with DSM 11798. This finding is inconsistent with another study, where minor effects of DON, such as a decreased albumin concentration at the end of the trial, were prevented by addition of DSM 11798 (Grenier et al. 2013).

The data from the plasma analyses of DON and its metabolites, especially that of DOM-1, confirmed that DSM 11798 was not able to reduce the epoxide group in DON in this *in vivo* study (Figure 2). The enzymatic reduction of DON to DOM-1 by DSM 11798 was expected to result in decreased DON plasma concentrations and increased DOM-1 plasma concentrations in pigs receiving

DON-contaminated diets supplemented with DSM 11798 compared to pigs fed contaminated diets without the detoxifying microorganism (Dänicke et al. 2004; Fuchs et al. 2000; Fuchs et al. 2002; Schatzmayr et al. 2006). However, the concentrations of both DON and DOM-1 (Figure 2) were not significantly different in the plasma of pigs fed contaminated diets supplemented with DSM 11798 compared with pigs fed diets without DSM 11798. On the other hand, the supplementation with DSM 11798 in the DON-contaminated feed resulted in significantly higher levels of DON–glucuronides (Figure 2c and Figure 2d). As no other studies we are aware of have included DON-glucuronides in investigations related to DSM 11798/BBSH 797, we cannot compare the result with previous research. At present, we have no explanation for the effect of DSM 11798 on the DON-glucuronide levels. However, presence of DSM 11798 in the gut might hypothetically influence enteric or bacterial ß-glucuronidase activity, and thereby change the enteric recycling of deoxynivalenol, resulting in increased DON-glucuronide levels in the systemic circulation (Yang et al. 2017).

Traditionally, DON-3-Glc and DON-15-Glc have been the main identified DON metabolites in non-ruminant mammals. Recent research has identified a range of additional metabolites in the urine of rodents and humans, including iso-DON-8-glucuronide, iso-DON-3-glucuronide, DOM-3-glucuronide and DON-3-sulfate (Schwartz-Zimmermann et al. 2017; Warth et al. 2016). In pigs, however, DON-3-Glc and DON-15-Glc still seem to be the dominating metabolites, and the iso-DON-glucuronides and DOM-glucuronides are found only in low concentrations (Schwartz-Zimmermann et al. 2017). In our experience, sulfate conjugation of DON is also of minor importance in pigs (unpublished results).

In this study, we found no differences in plasma concentrations of DON and its metabolites between male and female piglets. This is in contrast to the observations reported by Pestka et al. (2017) in mice, where they observed slower excretion and higher hepatic and renal concentrations of DON metabolites in male mice than in females.

The lack of activity of DSM 11798 to reduce the 12,13-epoxy group in DON and thereby detoxify the mycotoxin in the present study may have several explanations. One possible reason could be related to the feed production. In the present study, the experimental diets were prepared by a standard pelleting process. The heat involved in this process can impair the viability of bacteria. However, BBSH 797 has been proven to survive common pelleting process with pelleting temperatures up to 80 °C (European Food Safety Authority (EFSA) 2005; 2013), and the pelleting temperatures were held strictly at that level (Table 1). Furthermore, the survival of the DSM 11798 strain in our experimental diets was monitored after the pelleting process (Table 1) and was assessed to be sufficient to ensure effective detoxification.

It is possible that the impact of the pelleting process and the embedding of the DSM 11798 microorganism into a feed pellet themselves affect the microorganism's efficacy in pigs. Because the intestinal absorption of DON in pigs is fast, a delayed onset of the activity of the DSM 11798 in the pig gut may be sufficient to impair its efficacy against DON absorption and toxicity.

Another possibility for the lack of effect could be the stabilizing matrix used by Biomin in the preparation of the DSM 11798 product for our experiments, which was somewhat different from the commercial version. It should also be noted that the main feed ingredient in our study was DON-contaminated oats, while some of the other studies were based on contaminated maize. Further studies on the *in vivo* efficacy of DSM 11798 making use of an experimental design where both pelleted and meal diets are included are recommended.

In conclusion, in the present study, feeding pigs with pelleted, naturally DON-contaminated diets led to a transient effect on growth performance, which was significant in the first week of exposure. Some blood biochemical parameters were also influenced by the DON-contaminated diets. The inclusion of a feed additive containing the bacterial strain DSM 11798 in the pelleted feed was found to be ineffective both in the biotransformation of DON to less toxic components, such as DOM-1, and in preventing mycotoxin-related effects in the pigs fed the naturally DON-contaminated pelleted diets.

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Table titles

Table 1. Composition of the experimental diets.

Table 2. Effect of experimental diets on pig growth.

Table 2a. Growth performance results at the assessment stages in the study.

Table 2b. Probabilities derived from the fixed-effect tests (repeated measures).

Table 3. Daily DON doses in the pigs during the five-week exposure study.

Table footnotes

Table 1.

[a] Feed Additive (*Coriobacteriaceae* strain DSM 11798).

[b] FA dose in the finished pellets was determined with respect to an inert tracer substance in the DSM 11798 preparation.

Table 2a.

[a] *Coriobacteriaceae* strain DSM 11798.

[b] Mean ± standard deviation.

[c] ADG, average daily gain; [d] ADFI, average daily feed intake; [e] Feed conversion ratio (feed consumption/weight gain).

Means within a row with no common letter are significantly different ($p < 0.05$). The differences were evaluated by post-hoc Tukey test.

Results from weeks 3 and 4 (day 15-28) are omitted for space reasons. They are reported in the supplementary material (Table S2).

Table 2b.

[f] F-ratio and [g] *p*-values in the fixed-effect test table present the main effect and interactions.

Table 3.

[a] Standard deviation.

[b] Mean body weight in a week.

[c] Daily intake of DON in feed (mg day^{-1}): ADFI×DON conc. in feed.

[d] Daily DON dose (µg kg^{-1} body weight day^{-1}).

Figure captions

Figure 1. Effect of experimental diets on biochemistry parameters. Selected serum biochemical parameters of pigs over the course of the experiment according to DON levels (n = 12 in each group, pooled data for the main effect "DON levels", * $p < 0.05$). Each error bar shows the standard error of the mean.

Figure 2. Effect of experimental diets on plasma concentrations of DON (Figure 2a), DOM-1 (Figure 2b), DON-3-GlcA (Figure 2c) and DON-15-GlcA (Figure 2d). Plasma concentrations of DON and its metabolites (ng ml^{-1}) in pigs over the course of the experiment were dependent on DON levels and the addition of DSM 11798 (n = 6 in each group, pooled data for the main effect "DON levels and addition of DSM 11798"). The data points are the mean of plasma concentrations measured at three different time points per day. The first tick D0 (x-line) in each figure refers to the measurement on day 4 of acclimatisation. D1 baseline refers to the first measurement before the diet change at 08:00, and D1 refers to the average of plasma concentrations on two measurements after the diet changes at 11:00 and 16:00 on the first day of exposure. Error bars are the standard error of the mean.

Supplemental information.

Table titles

Table S1. Toxin contents in the experimental diets, as measured by multi-toxin LC-MS/MS.

Table S2. Growth performance results from days 15-28 in the feeding study (results omitted from Table 2 in the main text.)

Table footnotes

Table S1

[a] Feed Additive (*Coriobacteriaceae* strain DSM 11798).

Table S2

[a] *Coriobacteriaceae* strain DSM 11798.

[b] Mean ± standard deviation.

[c] ADG, average daily gain; [d] ADFI, average daily feed intake; [e] Feed conversion ratio (feed consumption/weight gain).

Figure captions

Figure S1. Average daily gain (g d^{-1}) of pigs over the course of the experiment in relation to DON levels [(n = 12 in each group), pooled data for the main effect "DON levels", * $p < 0.05$]. Each error bar is constructed using one standard error of the mean.

Figure S2. Average daily feed intake (g d^{-1}) of pigs over the course of the experiment in relation to DON levels [(n = 12 in each group), pooled data for the main effect "DON levels"]. Each error bar is constructed using one standard error of the mean.

	Experimental Diets								
Ingredients	**Control**	**Control + FA[a]**	**Low-DON**	**Low-DON + FA[a]**	**Medium-DON**	**Medium-DON + FA[a]**	**High-DON**	**High-DON + FA[a]**	**Unit**
Oats, uncontaminated	64.0	64.0	55.0	55.0	36.0	36.0	8.0	8.0	%
Oats, DON-contaminated	_	_	9.0	9.0	28.0	28.0	56.0	56.0	%
Wheat, uncontaminated	9.6	9.5	9.6	9.5	9.5	9.5	9.5	9.5	%
Fish meal	11.4	11.4	11.4	11.4	11.4	11.4	11.4	11.4	%
Soya extracted	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	%
Rapeseed cake Mestilla	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	%
Soya oil (raw)	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	%
Calcium carbonate (limestone)	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	%
Monocalcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	‰
Salt (NaCl)	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	‰
Mikromin. Swine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	‰
Selenpremix	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	‰
Normin ferrous fumarate	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	‰
Vitamin-A	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	‰
Vitamin-E V5	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	‰
Vitamin-ADKB	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	‰
Stay C 35%	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	‰
L-lysine	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	‰
DL-methionine	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	‰
L-threonine	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	‰
L-Valin	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	‰
L-Tryptophan	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	‰
Formic Acid 85%	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	‰
MAXAROME RP SWEET 1516	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	‰
PHYZYME XP 5000 TPT	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	‰
Deoxynivalenol	0.13	0.13	0.92	1.00	2.20	2.50	5.00	5.70	mg kg^{-1}
3-ac-DON	<0.01	<0.01	0.06	0.08	0.22	0.24	0.64	0.58	mg kg^{-1}
15-ac-DON	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	mg kg^{-1}
DON-3-Glc	<0.03	<0.03	0.10	0.10	0.41	0.38	0.64	0.52	mg kg^{-1}
***Coriobacteriacea* strain DSM 11798 (FA)[b]**	_	0.10	_	0.10	_	0.10	_	0.10	%
Viable cell Count		3.1×10^8	_	2.5×10^8	_	1.9×10^8	_	1.2×10^8	cfu kg^{-1}

Pelleting temprature	81.8	79.5	79.7	79.9	80.3	80.5	79.9	80.2	ºC

Table 1

	Diets					
	DON levels				Feed additive[a]	
	Control (n=12)[b]	Low-DON (n=12)[b]	Medium-DON (n=12)[b]	High-DON (n=12)[b]	FA (n=24)[b]	Non-FA (n=24)[b]
Pre-exposure (day -6 to day 0)						
Initial Body Weight (kg)	10.9 ± 1.2	10.9 ± 1.4	10.3 ± 1.8	11.9 ± 1.5	10.9 ± 1.5	11.1 ± 1.6
ADG (g day -1)[c]	251 ± 90	264 ± 80	213 ± 72	245 ± 96	233 ± 85	254 ± 85
ADFI (g day -1)[d]	338 ± 81	363 ± 82	303 ± 87	346 ± 91	323 ± 89	352 ± 82
FCR[e]	1.42 ± 0.25	1.43 ± 0.31	1.51 ± 0.51	1.52 ± 0.36	1.47 ± 0.32	1.48 ± 0.41
Final Body Weight (kg)	12.7 ± 1.6	12.8 ± 1.6	11.7 ± 2.1	13.6 ± 1.9	12.5 ± 1.9	12.8 ± 1.9
Exposure period (day 1-7)						
ADG (g day -1)	577 ± 141	**662 ± 117b**	532 ± 116	**437 ± 90a**	523 ± 144	581 ± 133
ADFI (g day -1)	640 ± 145	714 ± 127	571 ± 118	513 ± 84	574 ± 133	645 ± 139
FCR	1.13 ± 0.16	1.09 ± 0.17	1.08 ± 0.11	1.19 ± 0.13	1.12 ± 0.16	1.12 ± 0.13
Final Body Weight (kg)	16.7 ± 2.5	17.4 ± 2.1	15.5 ± 2.6	16.6 ± 2.3	16.2 ± 2.6	16.9 ± 2.2
Exposure period (day 8-14)						
ADG (g day -1)	650 ± 107	612 ± 83	582 ± 93	595 ± 120	595 ± 96	625 ± 107
ADFI (g day -1)	901 ± 221	922 ± 176	789 ± 165	801 ± 177	830 ± 187	876 ± 194
FCR	1.37 ± 0.15	1.50 ± 0.14	1.34 ± 0.11	1.35 ± 0.15	1.38 ± 0.09	1.39 ± 0.13
Final Body Weight (kg)	21.2 ± 3.1	21.7 ± 2.5	19.5 ± 3.2	20.8 ± 3.0	20.4 ± 3.2	21.3 ± 2.8
Exposure period (day 29-35)						
ADG (g day -1)	831 ± 141	883 ± 135	863 ± 138	818 ± 164	813 ± 151	885 ± 127
ADFI (g day -1)	1369 ± 267	1462 ± 263	1343 ± 278	1401 ± 264	1324 ± 287	1463 ± 223
FCR	1.65 ± 0.21	1.65 ± 0.12	1.55 ± 0.14	1.71 ± 0.16	1.62 ± 0.17	1.65 ± 0.17
Final Body Weight (kg)	37.6 ± 5.3	38.9 ± 3.8	35.6 ± 5.6	37.3 ± 4.7	36.4 ± 5.3	38.4 ± 4.2
Exposure period (day 1-35)						
ADG (g day -1)	714 ± 110	746 ± 89	686 ± 108	677 ± 99	681 ± 113	731 ± 85
ADFI (g day -1)	1121 ± 225	1195 ± 161	1055 ± 183	1083 ± 180	1064 ± 205	1162 ± 163
FCR	1.56 ± 0.11	1.60 ± 0.06	1.53 ± 0.07	1.59 ± 0.06	1.56 ± 0.08	1.58 ± 0.07

Table 2a

	Items and interactions						
	DON	FA	Time	DON × FA	DON × Time	FA × Time	DON × FA × Time
ADG (g day $^{-1}$)	F^{f} (3,40) =1.14, p^{g}=0.34	F (1,40) =2.84, p=0.09	**F (5,200) = 355.77, p <0.0001**	F (3,40) = 0.57, p= 0.63	**F (15,200) = 2.77, p= 0.0006**	F (5,200) = 0.65, p = 0.66	F (15,200) = 0.75, p = 0.72
ADFI (g day $^{-1}$)	F (3,40) =1.30, p=0.28	F (1,40) =3.32, p=0.07	**F (5,200)= 581.25, p <0.0001**	F (3,40) = 0.67, p= 0.57	F (15,200) = 1.17, p= 0.29	F (5,200) =1.82, p = 0.10	F (15,200) = 0.52, p = 0.92
FCR	F (3,40) =0.87, p=0.46	F (1,40) =0.97, p=0.32	**F (5,200)= 36.23, p <0.0001**	F (3,40) = 1.07, p= 0.37	F (15,200) = 0.86, p= 0.61	F (5,200) =0.24, p = 0.95	F (15,200) = 0.99, p = 0.45

Table 2b

		Exposure time				
		Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**
Diets		Mean ± SD [a] (*n*=6)	Mean ± SD [a] (*n*=6)	Mean ± SD [a] (*n*=6)	Mean ± SD [a] (*n*=6)	Mean ± SD [a] (*n*=6)
Control	Body Weight[b] (kg)	15.1 ± 1.9	19.8 ± 2.5	24.9 ± 3.1	30.5 ± 3.7	36.5 ± 4.2
	DON intake[c] (mg day^{-1})	0.10 ± 0.02	0.13 ± 0.03	0.14 ± 0.03	0.17 ± 0.03	0.19 ± 0.03
	DON dose[d]	6.3 ± 0.9	6.3 ± 0.8	5.6 ± 0.5	5.6 ± 0.3	5.2 ± 0.3
Control + FA	Body Weight[b] (kg)	14.2 ± 2.2	18.1 ± 3.0	22.6 ± 3.5	27.6 ± 4.1	32.9 ± 5.0
	DON intake[c] (mg day^{-1})	0.07 ±0.01	0.11 ± 0.03	0.12 ± 0.02	0.14 ± 0.03	0.16 ± 0.04
	DON dose[d]	5.0 ± 0.2	5.9 ± 0.6	5.4 ± 0.6	5.1 ± 0.7	5.0 ± 0.6
Low-DON	Body Weight[b] (kg)	14.8 ± 1.7	19.2 ± 2.2	23.9 ± 2.3	29.3 ± 2.3	35.2 ± 2.8
	DON intake[c] (mg day^{-1})	0.66 ± 0.10	0.84 ± 0.19	0.99 ± 0.04	1.17 ± 0.10	1.34 ± 0.17
	DON dose[d]	44.7 ± 4.5	43.5 ± 6.9	41.4 ± 4.3	40.0 ± 2.5	38.0 ± 2.3
Low-DON + FA	Body Weight[b] (kg)	15.1 ± 1.6	19.9 ± 2.6	24.8± 3.1	30.4 ± 3.6	36.4 ± 4.3
	DON intake[c] (mg day^{-1})	0.72 ± 0.16	0.93 ± 0.16	1.09 ± 0.16	1.30 ± 0.26	1.47 ± 0.35
	DON dose[d]	46.7 ± 6.4	47.2 ± 8.1	44.1 ± 5.0	42.7 ± 6.4	40.2 ± 7.3
Medium-DON	Body Weight[b] (kg)	14.5 ± 2.6	18.6 ± 3.2	23.2 ± 3.5	28.5 ± 4.2	34.4 ± 5.4
	DON intake[c] (mg day^{-1})	1.38 ± 0.26	1.80 ± 0.37	2.24 ± 0.23	2.74 ± 0.50	3.16 ± 0.68
	DON dose[d]	96.4 ± 22.0	96.4 ± 7.0	97.3 ± 10.0	96.1± 9.0	91.1 ± 9.1
Medium-DON + FA	Body Weight[b] (kg)	12.7 ± 1.8	16.5 ± 2.5	20.8 ± 3.0	25.7 ±3.9	29.4± 3.2
	DON intake[c] (mg day^{-1})	1.30 ± 0.28	1.91 ± 0.44	2.26 ±0.42	2.70 ± 0.54	3.14 ± 0.62
	DON dose[d]	103 ± 21	115 ± 11	108 ± 11	105 ± 8	107 ± 18
High-DON	Body Weight[b] (kg)	15.1 ± 2.1	18.8 ± 2.5	23.6 ± 3.1	29.1 ± 3.5	35.1 ± 3.8
	DON intake[c] (mg day^{-1})	2.59 ± 0.53	4.06 ± 0.74	5.07 ±0.83	6.71 ± 0.89	7.60 ±1.03
	DON dose[d]	172 ± 32	217 ± 31	215 ± 21	231 ± 23	217 ±22
High-DON + FA	Body Weight[b] (kg)	15.0 ± 2.4	18.6 ± 3.0	23.1 ± 3.6	28.3 ± 4.1	33.7± 5.1
	DON intake[c] (mg day^{-1})	2.90 ± 0.39	4.51 ± 1.23	5.42 ± 0.75	6.64 ± 1.58	7.32 ±1.63
	DON dose[d]	195 ± 26	238 ± 28	235 ± 16	233 ± 29	216 ± 16

Table 3

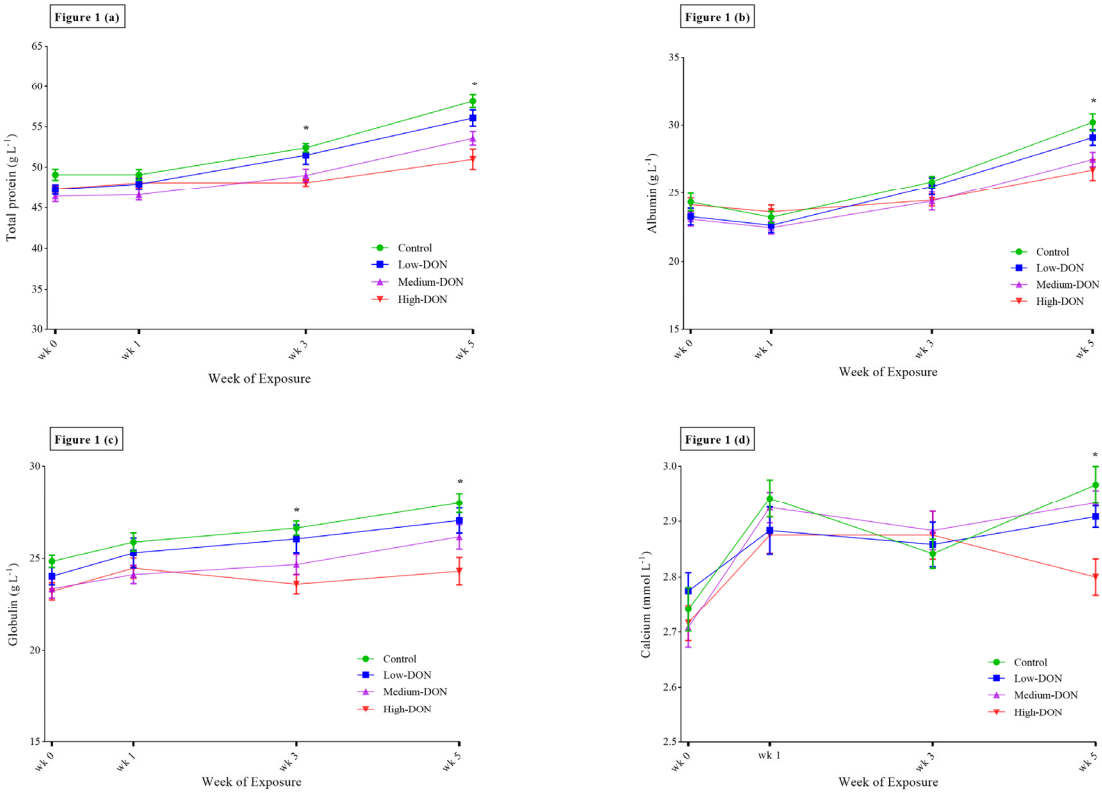

Figure 1

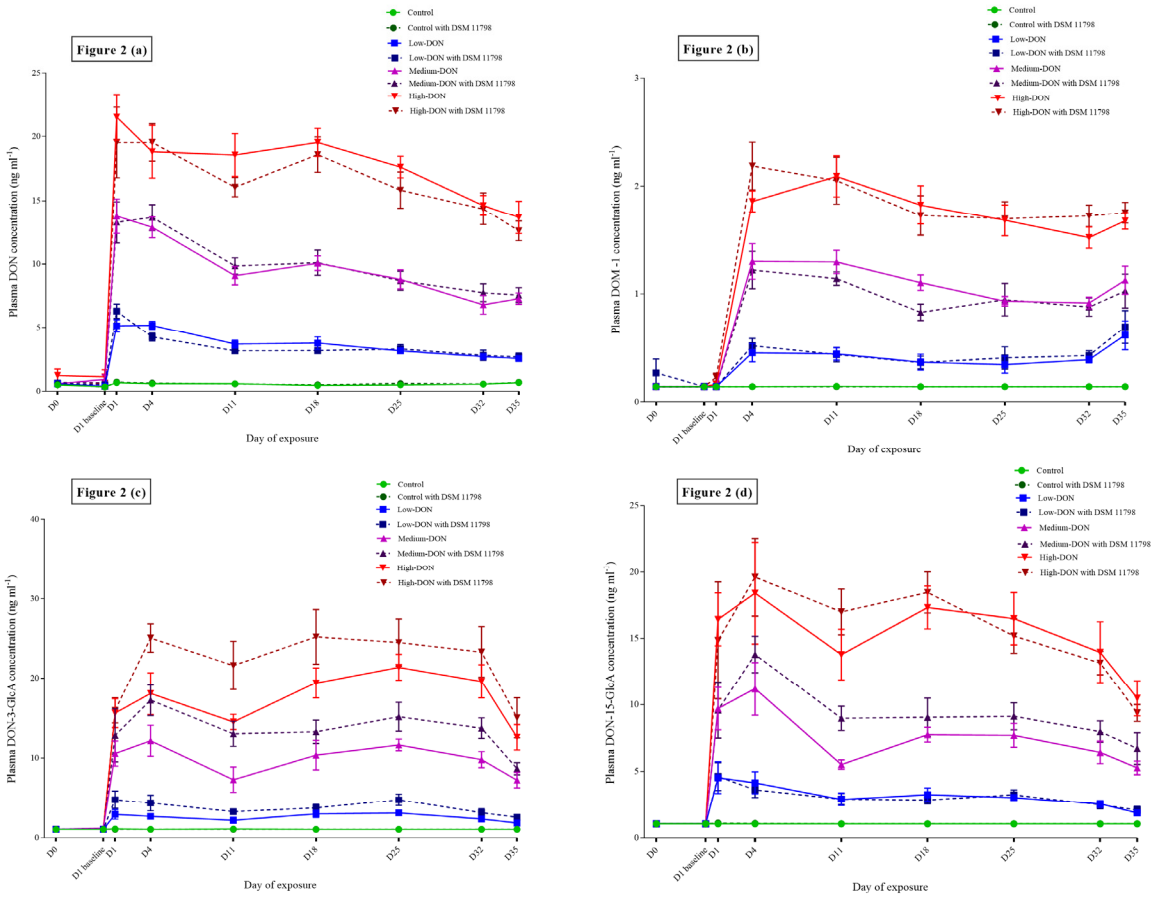

Figure 2

	Experimental Diets							
Mycotoxin (µg kg^{-1})	**Control**	**Control + FA[a]**	**Low-DON**	**Low-DON + FA[a]**	**Medium-DON**	**Medium-DON + FA[a]**	**High-DON**	**High-DON + FA[a]**
15-Hydroxyculmorinculmorin	20	< LOD	298	85	319	266	488	589
3-Nitropropionic acid	6.0	4.8	6.3	11.4	8.7	5.0	2.3	5.2
Alternariol	< LOD	< LOD	0.90	< LOD	< LOD	3.63	< LOD	2.70
Alternariolmethylether	0.45	0.33	0.41	0.37	< LOD	3.03	0.29	< LOD
Antibiotic Y	2207	874	1145	1318	1547	713	271	339
Apicidin	< LOD	0.9	4.8	13.6	16.0	18.3	28.2	31.2
Asperglaucide	61.5	54.0	56.4	54.5	55.5	54.3	55.2	56.5
Aurofusarin	59	67	46	63	62	53	257	190
Beauvericin	0.9	1.1	4.8	4.7	14.4	16.1	25.7	29.9
Brevianamid F	99	120	152	80	74	78	81	78
Butenolid	15.5	27.8	22.3	67.6	36.1	32.4	71.2	42.3
Chanoclavin	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.15	< LOD
Chrysogin	62	64	77	73	90	91	106	96
Culmorin	59	95	466	550	1426	1354	2346	2637
cyclo(L-Pro-L-Tyr)	286	322	397	278	253	268	285	242
Cytochalasin B	10.2	7.5	< LOD	< LOD	12.6	7.8	19.0	< LOD
Deoxynivalenol	136	211	828	895	2220	2284	4124	4097
DON-3-glucoside	16	12	65	74	196	204	423	391
Emodin	3.7	4.5	3.9	2.5	3.8	4.5	3.3	6.6
Enniatin A	2.2	2.4	1.8	1.9	1.3	2.0	1.1	1.3
Enniatin A1	6.3	6.7	6.9	7.2	5.5	9.6	7.2	8.3
Enniatin B	64	66	78	62	40	50	23	25
Enniatin B1	42	41	47	43	29	38	24	24
Enniatin B2	2.6	2.6	2.9	2.5	1.5	1.7	1.1	1.3
Enniatin B3	0.02	0.02	0.02	0.02	0.01	0.01	< LOD	0.01
Epiequisetin	0.50	0.18	0.22	0.15	0.09	0.27	0.25	0.14
Equisetin	1.12	0.30	0.40	0.36	0.30	0.37	0.06	0.21
Ergocornine	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	1.18	< LOD
Ergocorninin	0.21	< LOD	< LOD	0.30	0.29	0.31	0.90	0.56
Ergocristine	< LOD	37.03	< LOD	< LOD	< LOD	2.08	1.99	< LOD
Ergocristinine	< LOD	6.30	< LOD	< LOD	0.21	0.30	0.51	0.27
Ergocryptine	2.60	4.46	3.54	3.71	3.48	3.26	7.89	5.71
Ergocryptinine	< LOD	< LOD	< LOD	< LOD	1.08	< LOD	2.37	1.75
Ergometrinin	3.04	< LOD	7.41	3.82	< LOD	< LOD	< LOD	< LOD
Ergometrinine	0.32	0.30	0.41	0.25	0.31	0.20	0.25	0.36
Ergosin	< LOD	15.6	3.8	< LOD	1.0	1.0	1.6	3.0
Ergosinion	0.15	3.17	1.01	0.15	0.22	0.23	0.56	0.53
Ergotamine	< LOD	< LOD	42.82	< LOD	< LOD	< LOD	< LOD	< LOD
Ergotaminine	< LOD	< LOD	3.19	< LOD	< LOD	< LOD	< LOD	< LOD
HT-2 toxin	< LOD	< LOD	< LOD	< LOD	< LOD	6.04	< LOD	< LOD
Infectopyron	91	100	94	80	95	100	94	104

Lotaustralin	6.8	12.9	6.4	< LOD	5.6	5.1	2.9	3.3
Macrosporin	< LOD	0.42	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Moniliformin	8.4	12.2	9.0	9.1	5.9	6.4	3.6	3.5
Nivalenol	< LOD	< LOD	30	31	83	120	252	216
Rugulusovin	5.78	< LOD	< LOD	< LOD	< LOD	7.95	< LOD	6.86
Secalonic acid D	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	5.82	< LOD
T2-toxin	4.18	1.65	< LOD	< LOD	6.69	< LOD	< LOD	< LOD
Tentoxin	3.13	3.25	3.54	3.17	2.52	2.21	3.01	1.97
Zearalenone	0.7	1.3	2.6	4.5	9.9	11.5	23.8	21.0
Zearalenone-sulfate	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	2.40	< LOD

Table S1

	Diets					
	DON levels				Feed additive[a]	
	Control (n=12)[b]	Low-DON (n=12)[b]	Medium-DON (n=12)[b]	High-DON (n=12)[b]	FA (n=24)[b]	Non-FA (n=24)[b]
Exposure period (day 15-21)						
ADG (g day -1)[c]	720 ± 90	760 ± 110	704 ± 97	731 ± 69	706 ± 99	751 ± 85
ADFI (g day -1)[d]	1010 ± 204	1079 ± 109	959 ± 141	982 ± 146	974 ± 166	1041 ± 140
FCR[e]	1.39 ± 0.11	1.43 ± 0.11	1.37 ± 0.12	1.34 ± 0.11	1.38 ± 0.10	1.39 ± 0.13
Final Body Weight (kg)	27.1 ± 2.8	26.0 ± 3.3	26.2 ± 3.7	24.4 ± 3.6	26.5 ± 3.1	25.4 ± 3.7
Exposure period (day 22-28)						
ADG (g day -1)	792 ± 123	811 ± 127	750 ± 183	803 ± 149	767 ± 161	811 ± 127
ADFI (g day -1)	1203 ± 245	1287 ± 193	1162 ± 223	1252 ± 239	1164 ± 251	1289 ± 178
FCR	1.52 ± 0.17	1.59 ± 0.17	1.58 ± 0.23	1.56 ± 0.11	1.52 ± 0.17	1.60 ± 0.17
Final Body Weight (kg)	32.8 ± 3.2	31.7 ± 4.1	31.7 ± 4.3	29.6 ± 4.5	32.1 ± 3.6	30.8 ± 4.6

Table S2

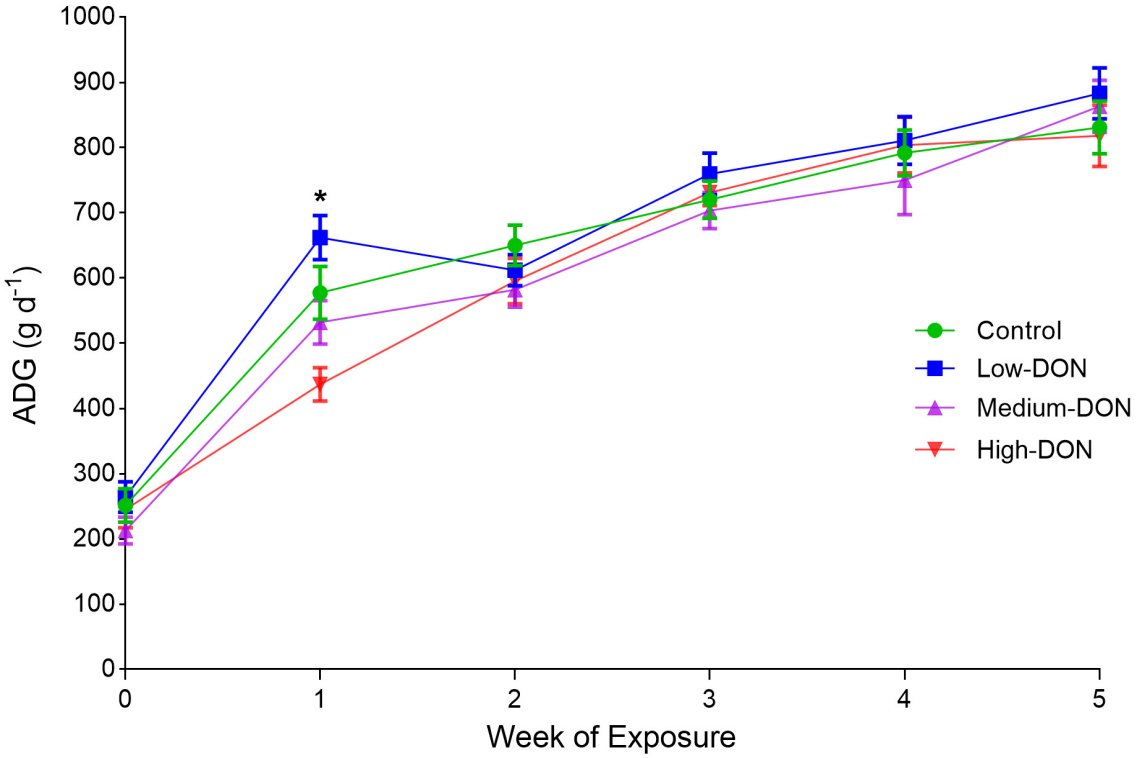

Figure S1

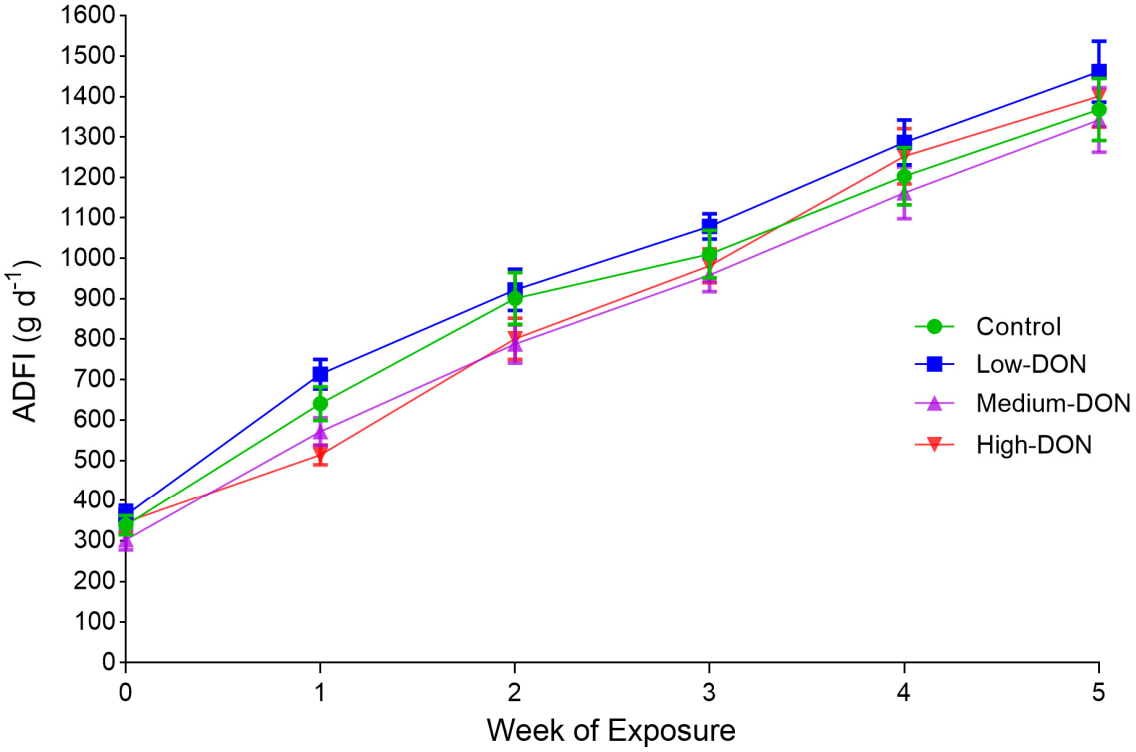

Figure S2