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In Vivo Contribution of Deoxynivalenol-3-β-D-glucoside to Deoxynivalenol Exposure in Broiler Chickens and Pigs: Oral Bioavailability, Hydrolysis and Toxicokinetics

Nathan Broekaert[†]*, Mathias Devreese[†2], Thomas van Bergen[‡2], Stijn Schauvliege[‡], Marthe De Boevre[#], Sarah De Saeger[#], Lynn Vanhaecke[⊥], Franz Berthiller[¤], Herbert Michlmayr[&], Alexandra Malachová[¤], Gerhard Adam[&], An Vermeulen[#], Siska Croubels[†]

[†] Ghent University, Faculty of Veterinary Medicine, Department of Pharmacology, Toxicology and Biochemistry, Salisburylaan 133, 9820 Merelbeke, Belgium

[‡] Ghent University, Faculty of Veterinary Medicine, Department of Surgery and Anaesthesia of Domestic Animals, Salisburylaan 133, 9820 Merelbeke, Belgium

[#] Ghent University, Faculty of Pharmaceutical Sciences, Department of Bioanalysis, Ottergemsesteenweg 460, 9000 Ghent, Belgium

[⊥] Ghent University, Faculty of Veterinary Medicine, Department of Veterinary Public Health and Food Safety, Salisburylaan 133, 9820 Merelbeke, Belgium

[¤] University of Natural Resources and Life Sciences, Vienna, Department IFA-Tulln, Christian Doppler Laboratory for Mycotoxin-Metabolism, Konrad-Lorenz-Str. 20, 3430 Tulln, Austria

[&] University of Natural Resources and Life Sciences, Vienna, Department of Applied Genetics and Cell Biology, Konrad-Lorenz-Str. 24, 3430 Tulln, Austria

* Corresponding author (Tel: +32 9 264 73 47; Fax: +32 9 264 74 97; E-mail: siska.croubels@ugent.be)

[2] shared second authorship

Abstract - Cross-over animal trials were performed with intravenous and oral administration of deoxynivalenol-3-β-D-glucoside (DON3G) and deoxynivalenol (DON) to broiler chickens and pigs. Systemic plasma concentrations of DON, DON3G and de-epoxy-DON were quantified using liquid chromatography-tandem mass spectrometry. Liquid chromatography coupled to high-resolution mass spectrometry was used to unravel phase II metabolism of DON. Additionally for pigs, portal plasma was analysed to study presystemic hydrolysis

and metabolism. Data were processed via tailor-made compartmental toxicokinetic models. The results in broiler chickens indicate that DON3G is not hydrolysed to DON *in vivo*. Furthermore, the absolute oral bioavailability of DON3G in broiler chickens was low (3.79±2.68%) and comparable to that of DON (5.56±2.05%). After PO DON3G administration to pigs, only DON was detected in plasma, indicating a complete presystemic hydrolysis of the absorbed fraction of DON3G. However, the absorbed fraction of DON3G, recovered as DON, was approximately 5 times lower than after PO DON administration, 16.1±5.4% compared to 81.3±17.4%. Analysis of phase II metabolites revealed that biotransformation of DON and DON3G in pigs mainly consists of glucuronidation, whereas in chickens predominantly conjugation with sulfate occurred. The extent of phase II metabolism is notably higher for chickens than for pigs, which might explain the differences in sensitivity of these species to DON. Although *in vitro* studies demonstrate a decreased toxicity of DON3G compared to DON, the species dependent toxicokinetic data and *in vivo* hydrolysis to DON illustrate the toxicological relevance and consequently the need for further research to establish a tolerable daily intake.

Keywords - modified mycotoxins, masked mycotoxins, Fusarium toxins, animal trials

Introduction

Mycotoxin contamination of food and feed has been classified by several authors as the most important chronic dietary risk for human and animal health. The *Fusarium* mycotoxin deoxynivalenol (DON) is one of the most frequently occurring mycotoxins, contaminating 56% of feed and feed raw materials worldwide (Schatzmayr and Streit 2013). Additionally, feed can be contaminated with modified forms of DON, such as deoxynivalenol-3-β-D-glucoside (DON3G), causing a possible underestimation of the degree of contamination.

Following a request from the European Commission, the risks to human and animal health related to modified forms of the *Fusarium* mycotoxins zearalenone, nivalenol, T-2 and HT-2 toxin and fumonisins were evaluated by the European Food Safety Authority Panel on Contaminants in the Food Chain (EFSA CONTAM Panel). The panel considered it appropriate to assess human exposure to modified forms of the various toxins in addition to the parent compounds, because modified forms may be hydrolysed into the parent compounds or released from the matrix during digestion. However, no risk assessment for modified forms of DON is currently available (EFSA CONTAM Panel 2014). Concerning acetylated forms of DON, 3-acetyl-deoxynivalenol (3ADON) and 15-acetyl-deoxynivalenol (15ADON), both ADONs were completely absorbed and presystemically hydrolysed to DON in pigs (Broekaert et al. 2015b). In chickens, for both ADONs a higher absorbed fraction was observed compared to DON, as well as a presystemic hydrolysis of 3ADON and 15ADON to DON of 100% and 75.4%, respectively (Broekaert et al. 2015b).

Recent occurrence data (2010-2014) for DON and DON3G have been reviewed for unprocessed cereals, such as wheat, maize, barley, oat, rye and spelt (Broekaert et al. 2015a). Observed incidence, average concentration and maximum detected concentration for DON and DON3G were 84%, 458 µg/kg, 27,088 µg/kg and 55%, 85 µg/kg, 170 µg/kg, respectively. DON3G concentrations exceeding those of free DON have been detected in some hard red spring wheat samples (Sasanya et al. 2008), which indicate its high prevalence.

The susceptibility of animal species to DON can be ranked in the following decreasing order: pigs > mice > rats > poultry ≈ ruminants (Pinton and Oswald 2014). The toxicity of DON depends on its ability to efficiently cross

biological barriers in humans and animals, such as the intestinal and the blood-brain barrier. At the cellular level, DON primarily inhibits protein synthesis. Consequently, fast dividing cells such as intestinal epithelial and immune cells will be more susceptible to the detrimental effects of DON (Maresca 2013; Pestka 2010; Rotter et al. 1996). The cellular toxicity is mainly attributed to the presence of the epoxide moiety (Pestka 2010; Ueno et al. 1973). This functional group allows DON, and trichothecenes in general, to bind to ribosomes, and inhibit peptide bond formation. Additionally, DON in lower doses causes a 'ribotoxic stress response', leading to proinflammatory responses (Pestka 2008). Presumably glucosylation of DON greatly reduces its toxicity. The phytotoxicity of DON and DON3G was studied by means of a wheat germ extract-coupled *in vitro* transcription/translation system. While 1 µM of DON significantly decreased translation by 63%, 5 µM and 20 µM of DON3G resulted in a translation decrease of only 3.1% and 8.0%, respectively (Poppenberger et al. 2003). In a growth test with algal cells, 80 µM of DON3G, unlike an equimolar amount of DON, did not significantly alter growth (Suzuki and Iwahashi 2015). Decreased toxicity has also been observed in mice, where DON3G was largely incapable of evoking cytokine or chemokine responses in the spleen of mice orally gavaged with 2.5 mg/kg body weight (bw), in contrast to DON, except for slight elevations in IL-1β mRNA expression (Wu et al. 2014). Recently, *in silico* analysis suggested that DON3G, unlike DON, was unable to bind to the ribosome peptidyl transferase centre (Pierron et al. 2015). Additionally, comparative cytotoxicity of DON and DON3G was assessed on both proliferative Caco-2 cells by means of quantitation of the ATP present. After 48 h exposure to DON, an IC_{50} of 1.3 µM (0.384 µg/mL) was observed in proliferative Caco-2 cells. In contrast, no cytotoxicity was observed with up to 10 µM of DON3G (Pierron et al. 2015).

Regarding the hydrolysis of DON3G to DON, different *in vitro* studies reported that DON3G was not hydrolysed to DON in the human upper gastrointestinal (GI) tract by means of acid, stomach and gut juice, or recombinant human cytosolic β-glucosidase (Berthiller et al. 2011; Dall'Erta et al. 2013; De Angelis et al. 2014; De Nijs et al. 2012). Regarding the human lower GI tract, some *in vitro* experiments indicate that hydrolysis can take place and that the intestinal microbiota composition and their abundance can play an important role (Abbott 2004; Berthiller et al. 2011; Dall'Erta et al. 2013; Gratz et al. 2013; Hattori and Taylor 2009). *In vitro* models do not take into account important physiological and anatomical factors within and between different animal species and humans, such as intestinal mucosa and luminal content composition (enzymes, microbiota), blood flow in internal organs and enterohepatic recirculation (Gonzalez-Arias et al. 2013). To estimate these factors and especially the interactions between them, species-specific *in vivo* trials remain mandatory. Versilovskis *et al*. (2012) reported a first DON3G *in vivo* study in which two rats were fed 25 µg of DON3G by oral gavage, followed by blood collection at 17 and 55 min post-administration. DON was detected in the stomach albeit at only 2% of the applied dose. This confirms the conclusions of the *in vitro* studies, i.e. hydrolysis of DON3G in the stomach is negligible. Further down in the GI tract, DON3G could only be detected at 2-3% of the applied dose in the small intestine and at 1-2% in the colon. This remarkable decrease of DON3G in the small intestine is probably due to the presence of intestinal β-glucosidases (Versilovskis et al. 2012). Nagl *et al*. (2012) orally administered DON and an equimolar dose of DON3G to six rats, followed by urine and faeces collection over a period of two days. After PO administration of DON3G, less than 4% of the administered dose was recovered in urine, of which 35% as DON, 33% as DON-glucuronide (DON-GlcA), 24% as de-epoxy-deoxynivalenol (DOM-1) and 8% as DON3G. The presence of urinary DON clearly demonstrates the hydrolysis upon ingestion. In comparison, after PO DON administration, 15% of the dose was recovered in urine as DON-GlcA, DON and DOM-1, indicating a relatively low oral bioavailability for DON3G compared to DON in rats. Analysis of faecal samples of rats dosed with DON3G revealed that the vast majority of the metabolites of DON3G were excreted as DON and DOM-1 (sum: 99.5 ± 0.4%). Only traces of DON3G were detected, indicating that intestinal microbiota are effective in hydrolysing this masked mycotoxin in rats (Nagl et al. 2012). Recently, the results in rat were supported by *in vivo* experiments performed in pig by the same research group (Nagl et al. 2014). After intravenous (IV) administration of DON3G, no DON was detected in plasma, indicating that systemic hydrolysis of DON3G is negligible. After oral administration of DON3G, 40.3% of the dose was recovered in urine of which 2.6% as DON3G, next to DON (21.6%), DON-15-glucuronide (DON15GlcA) (6.8%), DOM-1 (5.9%) and DON-3-glucuronide (DON3GlcA) (3.4%) (Nagl et al. 2014). Consequently, it was concluded that *in vivo*

cleavage of DON3G predominantly occurs in the (lower) digestive tract. Oral bioavailability was lower for DON3G than for DON, deduced from the recovered DON equivalent amounts in urine, namely 84.8% for DON and 40.3% for DON3G (Nagl et al. 2014). Moreover, the oral bioavailability for both compounds in pig was remarkably higher than in rat.

However, both study designs in rat and pig do not allow determination of the site of hydrolysis (GI microbiota, GI tissues, portal blood, liver, systemic blood). Sampling portal blood would offer a huge advantage as it would allow to differentiate between presystemic and systemic hydrolysis and therefore significantly contribute to the understanding of the (pre)systemic hydrolysis of DON3G. Transsplenic portal vein catheterisation in pigs has previously been described (Gasthuys et al. 2009) and was used to study the presystemic metabolisation of xenobiotics (Reyns et al. 2009). Therefore, portal vein catheterisation offers a valuable tool to study possible presystemic hydrolysis of DON3G in pigs.

Currently, literature reports regarding the toxicokinetics of DON3G in humans and other animal species, such as broiler chickens, are scarce. Warth *et al*. performed a study with a human volunteer consuming a diet naturally contaminated with 138 µg DON/day, 7 µg DON3G/day and 20 µg 3ADON/day. DON3G, in contrast to DON, could not be detected in urine (Warth et al. 2013). For broiler chickens no data is available, although poultry have a high exposure to DON3G given their mainly cereal based diet. The GI anatomy, physiology and distinct microbiota species composition and abundance of poultry further contribute to the relevance of performing research in these species.

The goal of this study was to determine the absolute oral bioavailability, the degree of hydrolysis and the main toxicokinetic parameters of DON3G in broiler chickens and pigs. Additionally portal vein catheterisation in pigs allowed determination of the site of presystemic hydrolysis.

Materials and methods

Standards, reagents and solutions

DON (>99% purity) was obtained from Fermentek (Jerusalem, Israel). DON3G was enzymatically synthesized, purified and verified using nuclear magnetic resonance (NMR) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Michlmayr et al. 2015). No remaining DON (<0.05%) was detected in the produced DON3G. DON and DON3G were dissolved in analytical-grade ethanol (EtOH) (Merck, Brussels, Belgium), yielding a stock solution of 10 mg/mL and were used for animal trials and analytical experiments. DOM-1 (50 µg/mL) and $^{13}C_{15}$-DON (stable isotopically labelled internal standard, IS, 50 µg/mL) stock solutions in acetonitrile were purchased from Sigma-Aldrich (Diegem, Belgium). All stock solutions were stored at ≤-15 °C. Individual working standard solutions of 10 µg/mL, used for the analytical experiments, were prepared by diluting the above stock solutions with UHPLC-MS grade acetonitrile (Biosolve, Valkenswaard, The Netherlands). All working standard solutions were stored at ≤-15 °C. Standard mixture working solutions containing DON, DON3G and DOM-1 of 1 and 0.1 µg/mL were prepared by mixing appropriate dilutions of the individual working standard solutions in UHPLC-MS acetonitrile, stored at 2-8 °C. Solutions of DON in acetonitrile have been reported to be stable for 24 months at room temperature (Widestrand and Pettersson 2001). Water, methanol and glacial acetic acid were of UHPLC-MS grade (Biosolve). Microfilters (GV-PVDF 0.22 µm) were obtained from Millipore (Overijse, Belgium).

Animal trials

Broiler chickens

Six broiler chickens (Ross 308) were purchased from the Institute for Agriculture and Fisheries Research (Melle, Belgium) at the age of 3 weeks, as hatched. Water and feed were given *ad libitum*. The pen was climate controlled with temperatures between 21 and 25 °C, a relative humidity between 40 and 60% and an applied light schedule similar to commercial installations (18 h light/6 h dark). After a one week acclimatisation period, the animals had an average bw ± standard deviation (SD) of 1400 ± 131 g, and three broiler chickens were treated with DON3G by IV bolus injection in the *vena basilica*, and three broiler chickens received DON3G PO by means of gavage in the crop. After a wash-out period of 1 day, a cross-over design was applied, which resulted in a PO and IV administration of DON3G in each of the six broiler chickens. Next, a second wash-out period of three days was respected, and the same broiler chickens were subjected to an identical two-way cross-over (IV and PO) study with DON. The administered doses were based on the EU maximum guidance level of 5 mg DON/kg poultry feed (European Commission 2006). Given an average feed consumption of 4-week-old broiler chickens of 100 g feed/kg bw/day, 500 µg DON/kg bw was administered. For DON3G, an equimolar dose of 774 µg/kg bw was administered. The calculated volume of stock solution (10 mg/mL in EtOH) was diluted with saline (0.9% NaCl) (VWR, Leuven, Belgium) up to a volume of 0.5 mL (IV) or 1 mL (PO). Feed was deprived 12 h before administration and until 3 h post administration (p.a.). After oral administration of the toxins, 1 mL of tap water was administered to flush the gavage syringe and tube. Blood (0.5-1 mL) was sampled from the leg vein (*vena metatarsalis plantaris superficialis*) by venipuncture at 0 min (before administration) and at 5, 10, 20, 30, 45, 60, 90, 120 and 180 min p.a. Blood samples were centrifuged (2851 x *g*, 10 min, 4 °C) and plasma was stored at ≤ -15 °C until analysis.

Pigs

For the pig study, six clinically healthy male pigs (11 weeks of age, 26.3 ± 1.8 kg bw) were individually housed and were provided feed and water *ad libitum.* Natural lighting was applied, the enclosure was climate controlled to keep the temperature between 20 and 24 °C and the relative humidity between 20 and 40%. After a one week acclimatisation period, double lumen catheters were surgically placed into the *vena jugularis* of all six pigs. Additionally, in four of the six pigs a single lumen catheter was placed into the *vena porta* to study presystemic hydrolysis and biotransformation. The surgical procedure was as previously described (Gasthuys et al. 2009). After a period of two days, the animals were treated with DON3G by IV bolus injection using one lumen of the double lumen jugular catheters, or PO by means of gavage in the stomach, in a two way cross-over design. After DON3G administration, an identical cross-over administration with DON was set up in the same animals and respecting a wash-out period of 24 h between treatments. Twelve h before administration of the mycotoxins, the animals were fasted until 4 h p.a. The administered doses were calculated as for the broiler chicken trial. The EU maximum guidance level in pig feed is 0.9 mg/kg DON (European Commission 2006). Pigs at 12 weeks of age consume on average 40 g feed/kg bw/day. This resulted in the administration of 36 µg DON/kg bw. For DON3G, an equimolar dose of 55.7 µg/kg bw was administered. The calculated amount of toxin for each animal, dissolved in EtOH at 10 mg/mL, was diluted with saline to a volume of 1 mL (IV) or 10 mL (PO). After oral administration of the toxins, 50 mL of tap water was administered to flush the gavage syringe and tube. Blood (1-2 mL) was sampled via the other lumen of the jugular catheter and the *vena porta* catheter at 0 min (before administration) and at 5, 10, 20, 30, 45, 60, 90, 120, 240, 360 and 480 min p.a. Blood samples were centrifuged (2851 x *g*, 10 min, 4 °C) and plasma was stored at ≤ -15 °C until analysis.

The chicken and pig animal trials were approved by the Ethics Committee of the Faculty of Veterinary Medicine and Bioscience Engineering of Ghent University (EC2013/64 and EC2015/14, respectively).

Plasma sample preparation

Blank plasma was obtained by the collection of heparin treated blood from six different broiler chickens and pigs. The animals were fasted 12 h prior to blood collection. Plasma was obtained by centrifugation (2851 x *g*, 10 min, 4 °C) of the blood. The blank plasma was pooled, homogenised and stored at ≤ -15 °C until use for the preparation of matrix-matched calibrators and quality control samples.

Calibrator and quality control samples. To 250 µL of blank plasma, 25 µL of a 100 ng/mL IS working solution ($^{13}C_{15}$-DON) and appropriate volumes of the standard mixture working solutions (1, 10 100 and 1000 ng/mL) were added to obtain calibrator samples with mycotoxin concentrations of 0.1, 0.5, 1, 5, 10, 50, 100 and 500 ng/mL. After vortex mixing, acetonitrile was added up to a volume of 1 mL to precipitate plasma proteins. The samples were vortex mixed again, followed by a centrifugation step (10 min at 8517 x *g*, 4 °C). The supernatant was transferred to a new tube and evaporated to dryness under nitrogen at 45 °C. The dry residue was then redissolved in 200 µL of UHPLC-MS grade water, micro-filtrated and 10 µL was injected onto the LC-MS/MS instrument.

Incurred samples. To 250 µL of plasma, 25 µL of a 100 ng/mL IS working solution were added. After vortex mixing, the samples were subjected to the same sample preparation procedure as the calibrator samples.

Blank samples. After the addition of 750 µL of acetonitrile to 250 µL of blank plasma, the samples were extracted in the same way as the calibrator samples.

LC-MS/MS analysis and validation

Separation of DON, $^{13}C_{15}$-DON, DON3G and DOM-1 was achieved on a Hypersil Gold (reversed-phase) (50 x 2.1 mm i.d., 1.9 µm) column with a guard column (10 x 2.1 mm i.d., 5 µm) of the same type. Chicken and pig plasma were analysed on an Acquity UPLC® system coupled to a Xevo® TQ-S MS instrument (Waters, Zellik, Belgium).

All compounds were eluted with a gradient of water containing 0.3% glacial acetic acid (mobile phase A) and methanol containing 0.3% glacial acetic acid (mobile phase B), at a flow rate of 300 µL/min. The following gradient elution program was run: 0-1 min, 5% B; 1.0-1.1 min, linear gradient to 12% B; 1.1-5.0 min, 12% B; 5.0-5.1 min, linear gradient to 50% B; 5.1-8.0 min, 50% B; 8.0-8.1 min, linear gradient to 95% B; 8.1-10.0 min, 95% B; 10.0-10.1 min, linear gradient to 5% B; 10.1-12.0 min, 5% B. The MS was operated in the multiple reaction monitoring (MRM) mode with two ion transitions for each target analyte as presented in **Table 1**. Instrumental and compound specific parameters were optimized by the direct infusion of 10 ng/mL standard solutions in methanol/ water (50/50, v/v) + 0.3% glacial acetic acid with a flow rate of 10 µL/min. For the Xevo TQ-S, the desolvation gas flow rate was fixed to 800 L/h with a temperature of 550 °C, the cone gas flow rate was set at 150 L/h, capillary voltage was optimised at 3.0 kV and a collision gas flow of 0.15 mL/min was used. Dwell times of 44 - 52 ms/transition were chosen. Compound specific MS parameters, together with precursor and product ions used for quantification and qualification, are given in **Table 1**. DON, $^{13}C_{15}$-DON and DOM-1 were detected in positive electrospray ionisation mode (ESI+) as the protonated precursor ion $[M+H]^+$, DON3G was measured in ESI- as $[M+CH_3COO]^-$ adduct.

Given the unavailability of reference materials, validation was performed on fortified blank pig and broiler plasma samples. Both recommendations as defined by the European Community (Commission Decision 2002; Heitzman 1994) and the Veterinary International Conference on Harmonisation (VICH 2009) served as validation guidelines. The developed method was single laboratory validated.

Linearity of the response of the compounds was assessed by means of three matrix-matched calibration curves consisting of eight calibration points in the range of 0.1-500 ng/mL. The correlation

coefficients (r) and goodness-of-fit coefficients (gof) were determined, limits were set at ≥0.99 and ≤20%, respectively.

Within-day accuracy & precision were determined by analyzing six samples fortified at a low concentration level (limit of quantitation (LOQ) of the compounds) and at a high concentration level (100 ng/mL). Values for the relative standard deviation (RSD) could not exceed 2/3 of the $RSD_{max,}$ calculated according to the Horwitz equation, i.e. $RSD_{max} = 2^{(1-0.5\log Conc)} \times 2/3$. The acceptance criteria for accuracy were: −30% to +10% and −20% to +10% for concentrations between 1 and 10 ng/mL, and ≥10 ng/mL, respectively.

Between-day accuracy & precision were assessed by analyzing the low and high concentration levels in threefold on three consecutive days (n=3x3). The acceptance criteria for accuracy were identical to the values given above and RSD values could not exceed $RSD_{max}= 2^{(1-0.5\log Conc)}$.

The *LOQ* was calculated as the lowest concentration for which the method had acceptable results with regards to accuracy and precision. It was determined by spiking six blank plasma samples. The LOQ was also established as the lowest point of the calibration curve. The *limit of detection* (*LOD*) was calculated using the samples spiked at the LOQ level (n=6) corresponding to the concentration that could be determined with a signal-to-noise (S/N) ratio of 3.

Carry-over was evaluated by analysing a mixture of mobile phase A and B (50/50; v/v) directly after the highest calibrator (500 ng/mL).

The *specificity*, the capability of the method to distinguish signals of the analytes from any other substances or interferences, was determined on six blank plasma samples. For an acceptable specificity the S/N ratio of possible interfering peaks with similar retention times in these samples could not exceed the S/N ratio of 3.

Recovery and matrix effects. Two types of matrix-matched calibration curves were prepared by spiking blank calibrator samples before and after extraction. A third calibration curve was prepared in standard solution. All curves consisted of eight calibration points in the range of 0.1-500 ng/mL. The slopes of these calibration curves (external calibration, without IS) were compared to calculate the apparent recovery (R_A = 100 × slope spiked before extraction/slope standard solution), the matrix effect denoted as signal suppression/enhancement (SSE = 100 × slope spiked after extraction/slope standard solution) and the recovery of the extraction step (R_E = 100 × *slope spiked before extraction/*slope spiked after extraction). Regarding SSE, values ≤ 1 indicate ion suppression due to matrix effect, values ≥ 1 indicate ion enhancement (Matuszewski et al. 2003).

High resolution mass spectrometry

To determine the presence of phase II metabolites, i.e. glucuronide and sulfate conjugates, high resolution mass spectrometry (HRMS) analysis was performed on pig and broiler chicken plasma samples from three animals after IV and PO administration for identification and semi-quantification of DON3G, DON and its metabolites. The methodology was similar as in a previously described study (Devreese et al. 2015). Sample treatment, HPLC column and mobile phases were identical to the LC-MS/MS analysis. However, a different gradient elution program was applied: 0-1.0 5% B, 1.0-4.0 linear to 80% B, 4.0-6.7 min 80% B, 6.7-7.0 linear gradient to 5% B, 7.0-10.0 5% B. The (U)HPLC consisted of an Accela type 1250 High Speed LC and autosampler coupled to an Exactive Orbitrap HR mass spectrometer, equipped with a heated electrospray ionisation (HESI) probe operating in the negative ionisation mode (all from Thermo Fischer Scientific, Breda, The Netherlands). Accurate masses for DON ($[M+CH_3COO]^-$), DOM-1 ($[M+CH_3COO]^-$), DON-3-sulfate and DON3GlcA, 355.1414,

339.2016, 375.0755 and 471.1508, respectively, were based on Wan et al. and Devreese et al. (Devreese et al. 2015; Wan et al. 2014). Accurate mass of DON3G ($[M+CH_3COO]^-$) was determined to be 517.19250 by direct infusion of a 10 µg/mL solution in mobile phase A and B (50/50, v/v).

Toxicokinetic modeling and statistical analysis

Toxicokinetic analysis on the LC-MS/MS data was performed using WinNonlin Professional version 5.2.1. (Pharsight, St-Louis, MO). Plasma concentrations below the LOQ were not taken into account. For the toxicokinetic analysis, all values were recalculated to their molar concentrations, expressed as nmol/mL. For the analysis of DON IV and PO data in chickens and pigs, and for DON3G IV and PO in chickens, a tailor-made two-compartmental model (central compartment (Vc) and peripheral compartment (Vp)) with first order absorption and first order elimination kinetics was applied. For the IV and PO administration of DON3G in pigs, a tailor-made one-compartmental model was fitted. Due to a lag time after PO DON3G administration to pigs, only terminal concentration points were above the LOQ. Consequently, it was not possible to calculate toxicokinetic parameters for the individual animals, and these parameters have been calculated simultaneously on all pooled pig PO DON3G data. As a result, no standard deviations could be calculated, instead coefficients of variation of the models' predicted versus observed concentrations are provided.

In **Figure 1** and **2** a graphical representation of the constructed model is given for DON (IV & PO) and DON3G (IV & PO) in broiler chickens and pigs, respectively.

A $1/\hat{y}$ weighing was applied for all calculations with both the chicken and pig data. For all the described models, the Gauss-Newton (Levenberg and Hartley) algorithm was used with a maximum of 50 iterations.

The following primary and secondary toxicokinetic parameters were calculated for DON and DON3G after IV and PO administration: absorption rate constant (ka), intercompartmental flow (Q), total body clearance (CL), central volume of distribution (Vc), peripheral volume of distribution (Vp). FRAC is the absorbed fraction of the dose in either form (DON or DON3G). F indicates the absolute oral bioavailability, i.e. the fraction of DON or DON3G absorbed in the systemic circulation in its unchanged form. When both a central and peripheral volume of distribution were observed, the disposition rate constant (β) was calculated by dividing the Cl by the sum of Vc and Vp for each animal. When only a central volume of distribution was used, the elimination rate constant (ke) was calculated by dividing the Cl by the Vc for each animal. Presystemic hydrolysis (Pres. Hydr.) was the percentage of the total dose of DON3G that is hydrolysed presystemically to DON and absorbed as DON (%). Additionally, for PO data the descriptive toxicokinetic parameters maximal plasma concentration (C_{max}) and time to C_{max} (t_{max}) were given for DON. Elimination half lives ($t_{1/2el}$) were calculated as 0.693/β or 0.693/ke.

Statistical analysis of FRAC, F, C_{max} and t_{max} after DON3G administration compared to DON administration consisted of one-way ANOVA with post-hoc Scheffé tests (p value < 0.05) for both animal species (SPSS 20.0, IBM, Chicago, IL). Equality of variances criterion was determined by the Levene's test for homogeneity of variances (p value > 0.01).

Results and discussion

LC-MS/MS method validation

For the calibration model a linear, 1/x weighed, fitting was applied. The results for linearity (*r* and *gof*) and sensitivity (LOD and LOQ) are given in **Table 2**. The results for linearity were all in accordance with the acceptance criteria, with $r \geq 0.99$ and $gof \leq 20\%$. For chicken plasma, the LOD varied from 0.11 (DOM-1) to 0.47 (DON) ng/mL, whereas the LOQ was 1 ng/mL for all compounds. For pig plasma, LOD values ranged from 0.01 (DON) to 0.28 (DOM-1) ng/mL, LOQ values were 0.1 (DON3G), 0.5 (DON) and 1 (DOM-1) ng/mL.

The results for matrix effects (SSE), apparent recovery (R_A) and extraction recovery (R_E) are also depicted in **Table 2**. SSE values for all mycotoxins varied from 44 to 112%, which demonstrated the need for matrix-matched calibration curves for a correct quantification. The matrix effects could be diminished by further optimizing the sample cleanup. However, a more elaborate cleanup tends to diminish the R_E, possibly limiting the sensitivity of the method. Values for R_E varied between 68.2 and 97.0% and R_A values ranged from 41.8 to 76.2%, with the majority of the latter deviation caused by SSE.

Table 3 displays the results for accuracy (%) and precision (RSD, %). All results for chicken as well as pig plasma were within the acceptable ranges. The specificity test demonstrated that no interfering peaks were detected in the chromatographic elution zone of the analytes with S/N values ≥ 3 (results not shown).

Carry-over was evaluated and for none of the compounds signals were detected that could interfere with the response/area of the analytes at their given retention time (results not shown).

Toxicokinetic analysis

The goal of this study was to determine the absolute oral bioavailability of DON3G and DON, the degree of *in vivo* hydrolysis of DON3G to DON (differentiating between presystemic GI, presystemic portal, presystemic hepatic and systemic hydrolysis) and the toxicokinetic parameters of both toxins in broiler chickens and pigs. A tailor-made compartmental toxicokinetic model was developed which offers the advantage, compared to non-compartmental analysis, that for a given compound, PO and IV data can be fitted simultaneously for each animal allowing more reliable estimates as more data is available (Broekaert et al. 2015b).

For broiler chickens, no adverse effects were observed during the animal trial following PO and IV bolus administration of the mycotoxins. Similarly, no adverse effects were observed during the pig trial following IV and PO DON3G administration and PO DON administration. Following IV dosing of DON, two out of six pigs vomited within 0.5 h p.a. However, this had no observable influence on the results as these pigs were IV dosed.

Plasma concentration-time profiles for DON3G and DON in broiler chickens and pigs are presented in **Figure 3**. Each profile represents the mean of six animals ± SD for systemic plasma concentrations. Additionally for pigs, *vena porta* plasma concentration-time profiles are presented as mean of four animals ± SD. A first observation is the absence of DOM-1 in all analysed samples. The derived toxicokinetic results are shown in **Table 4**.

The FRAC of PO administered DON and DON3G to chickens and PO administered DON to pigs was calculated using the tailor made model. However, the FRAC of DON3G to pigs could not be calculated by this model as after PO administration of DON3G only DON was recovered and after IV administration only DON3G. The FRAC was therefore calculated manually by correcting the area under the curve (AUC) of DON3G after IV administration by multiplying this value with its Cl, and by correcting the AUC of DON after PO DON3G dosing

again by multiplying this value with its Cl and by correcting this ratio for the difference in molar masses, as shown in the formula below:

$$FRAC_{DON3G} = \frac{AUC_{DON\ after\ DON3G\ PO} * Cl_{DON\ after\ DON} * MM_{DON3G}}{AUC_{DON3G\ after\ DON3G\ IV} * Cl_{DON3G\ after\ IV\ DON3G} * MM_{DON}}$$

The mean (±SD) absorbed fractions of DON and DON3G in broiler chickens were 5.56±2.05% and 3.79±2.68%, respectively. Moreover, for DON and DON3G in chickens, F was equal to FRAC as DON and DON3G are absorbed in their unchanged form. The FRAC of DON in pigs, 81.3±17.4% was also identical to the F. The FRAC of DON3G in pigs was 16.1±5.4%. However, as after PO DON3G administration only DON was recovered, F equals 0, indicating complete hydrolysis of the absorbed fraction. The higher observed FRAC values for pigs compared to chickens is an important contributing factor to the decreased sensitivity of the latter species, and may be partially caused by its rapid GI transit time, leading to a reduced time for absorption in chickens.

After IV administration of DON3G to chickens and pigs, no hydrolysis to DON could be observed, indicating the absence of systemic hydrolysis for both species. For pigs, this confirms the findings of Nagl et al. (2014). After PO administration of DON3G to chickens, hydrolysis was not observed either. In contrast, after PO DON3G administration to pigs, only DON was recovered, indicating a complete presystemic hydrolysis of the absorbed fraction. Presystemic hydrolysis can take place at the level of GI microbiota, GI tissues, portal blood or the liver. To further determine the site of presystemic hydrolysis, blood was collected from the porcine *vena porta*, which drains the venous intestinal blood to the liver first. Interestingly, only DON was recovered and no DON3G was detected in these portal plasma samples after PO DON3G administration. This absence of DON3G in portal plasma as well as the absence of DON after IV DON3G administration, indicates that hydrolysis occurs at the site of the GI tract (microbiota or GI tissues) and not in portal blood nor in the liver. This is in accordance with previously reported *in vitro* results which reported that hydrolysis could be caused by intestinal microbiota and which have demonstrated the important role of microbiota composition and the abundance in the intestinal tract (Abbott 2004; Berthiller et al. 2011; Dall'Erta et al. 2013; Gratz et al. 2013; Hattori and Taylor 2009). Furthermore, β-glucosidase from humans and almonds proved incapable of DON3G hydrolysis to DON, a typical trait of micro-organisms (Berthiller et al. 2011). The observed lag time for DON absorption after PO administration of DON3G to pigs, 83.6 min, further supports the hypothesised intestinal hydrolysis of DON3G. This is also reflected in the significant higher t_{max} value of DON after PO DON3G administration in pigs. DON is mainly absorbed in the proximal part of the small intestine by means of passive diffusion. A less effective passive non-ionic diffusion is expected for DON3G as it is more polar and has a less favourable log D value (-2.74) compared to DON (-0.97) at both physiological and acidic pH values (Maresca 2013). The pH in the stomach and duodenum of adult pigs is 4.4 and 6.4-7.4, respectively (Merchant et al. 2011). These rather low pH values are unfavourable for most GI commensal bacteria. When moving to more distal parts of the small intestine, the pH increases and a more abundant growth of microbiota is observed (Maresca 2013). Consequently, the probability of DON3G hydrolysis to DON is increased, resulting in the observed lag time of 83.6 min. As chickens have a high bacterial load at the crop, thus before the small intestine, which is the main site of absorption, hydrolysis of DON3G to DON by GI microbiota would be expected after PO administration to chickens. For pigs, where the high bacterial load is located after the site of absorption, only limited hydrolysis to DON is expected. Remarkably, our observations were the opposite of these theoretical expectations. This discrepancy likely finds its cause in the bacterial species composition and abundance in both the chicken crop and pig distal small and large intestine.

The microbiota of pigs and chickens consist mainly of the phyla *Firmicutes* and *Bacteroidetes,* accounting for approximately 90% and 80% of the relative abundance, respectively (Kim and Isaacson 2015; Waite and Taylor 2015). The crop, anterior small intestine, duodenal and jejunal epithelial cells, and digesta of chicken are dominated by *Lactobacillus* spp. (Watkins and Kratzer 1983). The hydrolytic capacity of *Lactobacillus* spp. towards DON3G has been described (Berthiller et al. 2011). Of the tested *L. amylovorus*, *crispatus, fermentum, gasseri, paracasei, rhamnosus* and *plantarum,* only the latter was able to hydrolyse DON3G to DON, more specifically 34% after 4h incubation. This *L. plantarum* has been found in the GI tract of chickens, however it does not belong to the most abundant GI *Lactobacillus* species (Hilmi et al. 2007), in contrast, it is commonly found in human and other mammalian gastrointestinal tracts (de Vries et al. 2006). The hydrolytic capacities towards DON3G of *Enterococcus*, a large genus within *Firmicutes* species, has also been investigated. Of the seven tested species, *E. avium* was the only species unable to hydrolyse DON3G to DON. *E. casseliflavus, durans, faecalis, faecium, gallinarum* and *mundtii* all hydrolysed DON3G varying between 2% and 39% after a 4 h incubation (Berthiller et al. 2011). The occurrence of both *Enterococcus* species has been described in the GI tract of both chickens and pigs. Possibly, the relative amounts of *Enterococcus* and *Lactobacillus* species in the GI microbiota could partially explain our *in vivo* findings. However, the GI microbiota of animals consists of a multitude of phyla and genus, demonstrating interspecies as well as intraspecies differences, and differing between intestinal sections, providing a multitude of options for the observed differences in hydrolysis between chickens and pigs.

The mean (±SD) ka_{DON} values in chickens and pigs were comparable, 0.0315±0.0173/min and 0.0274±0.0107/min, respectively. For pigs ka_{DON} values of 0.00995±0.00292/min (Eriksen et al. 2003), 0.0310±0.0238/min (Goyarts and Dänicke 2006) and 0.0164±0.0161/min (Broekaert et al. 2015b) have been reported, and for chickens a ka_{DON} of 0.0203±0.0101/min (Broekaert et al. 2015b) was previously described. The ka_{DON3G} in chickens was slightly lower than that of DON, 0.0238±0.0203/min. For pigs no ka_{DON3G} could be calculated as it was fully hydrolysed to DON after PO administration. The resulting ka_{DON} after PO DON3G administration was 0.00501/min, significantly lower than the ka_{DON} after DON administration. The ka_{DON} after PO DON3G administration is influenced by presystemic hydrolysis, consequently this value is also determined by biotransformation in addition to absorption.

With regards to clearance, mean (±SD) Cl_{DON3G} values of 11.4±1.2 and 3.17±0.81 mL/min/kg were obtained for chickens and pigs, respectively. Cl_{DON} values in chickens and pigs were 88.8±12.4 and 5.25±1.25 mL/min/kg, respectively. For chickens, a comparable Cl_{DON} of 120 mL/min/kg (Osselaere et al. 2013) and 102±12 mL/min/kg (Broekaert et al. 2015b) have previously been described. Cl_{DON} values in pigs of 9.30±3.95 mL/min/kg (acute PO) (Goyarts and Dänicke 2006), 5.30±1.64 mL/min/kg (chronic PO) (Goyarts and Dänicke 2006), 15.1±3.8 mL/min/kg (acute PO) (Broekaert et al. 2015b) and a Cl_{DON}/F of 5.0±0.7 mL/min/kg (acute PO) (Devreese et al. 2014) have been reported, which are in the same range as the results in this study. The obtained mean (±SD) Vc_{DON} and Vp_{DON} values for chickens were 1350±578 and 1087±211 mL/kg, respectively. For pigs, Vc_{DON} and Vp_{DON} values were comparable, i.e. 614±127 and 789±486 mL/kg respectively. For chickens Vc_{DON3G} and Vp_{DON3G} values of 290±188 and 284±167 mL/kg were obtained. A Vd_{DON3G} in pigs of 249±67 mL/kg was obtained using a one-compartmental model. The observed lower Vd values for DON3G indicate that DON3G is more confined to intravascular fluids than DON, which can be theoretically explained by its increased polarity compared to DON, resulting in less effective passive non-ionic diffusion into tissues.

The mean (±SD) elimination half-lives ($t_{1/2el}$) for DON in broilers and pigs were 18.8±2.1 min and 163±59 min (2.71 h), respectively. For pigs, $t_{1/2el}$ of 5.3±2.4 h (acute PO, two-compartmental model) (Goyarts and Dänicke 2006), 2.4±0.7 h (acute PO, one-compartmental model) (Eriksen et al. 2003), 2.7±0.5 h (acute PO, one-compartmental model) (Devreese et al. 2014) and 2.9 h (acute PO, two-compartmental model) (Broekaert et al. 2015b) were observed. For broiler chickens comparable elimination half-lives of 27.9±6.9 min (IV) and

38.2±11.2 min (acute PO) (Osselaere et al. 2013) and 17.9 min (acute PO) (Broekaert et al. 2015b) were also reported. The $t_{1/2elDON3G}$ values for chickens and pigs were 34.0±6.2 min and 54.1±3.0 min, respectively. Remarkably, the $t_{1/2elDON3G}$ compared to that of DON was increased for chickens and decreased for pigs, highlighting the difference between both species with respect to DON3G's elimination. For pigs, this is mainly attributed to a lower Vd. For broiler chickens, additional to a lower Vd a reduced Cl was observed.

HRMS

The plasma concentration-time profiles of phase II metabolites are depicted in **Figure 4** and **Figure 5** for pigs and chickens, respectively. Since only semi-quantitative analysis was performed, absolute peak areas obtained on the HRMS instrument are depicted. For pigs, a first observation is that glucuronidation patterns after PO administration do not differ between systemic (*vena jugularis*) and presystemic (*vena porta*) plasma. This indicates that glucuronidation does not occur presystemically to a significant rate and extent. This hypothesis is further supported by practically identical observed mean glucuronidation ratios (area of DON-GlcA/ area of DON) after IV and PO administration, 4.94 and 4.98, respectively. For pigs, no sulfate conjugates were detected, in agreement with the previously reported lack of sulfation reactions in this species (Riviere and Papich 2013). In contrast chickens predominantly formed DON-3sulfate. The mean DON-3-sulfate/DON ratios in chickens after IV and PO DON administration were 392 and 9,890, respectively. The observed ratios are comparable to previously found values for IV and PO administration of DON to chickens of 243-453 and 1,365-29,624, respectively (Devreese et al. 2015). This remarkable contrast in ratio between IV and PO administration, points towards a presystemic sulfation of DON, either by GI microbiota, GI tissues or a presystemic liver effect. In chickens, no glucuronidation was observed. The extensive and rapid sulfation in chickens might partially explain the relative resistance of this species towards the detrimental effects of DON.

In conclusion, broiler chickens do not hydrolyse DON3G to DON *in vivo.* Additionally, DON3G has a low absolute oral bioavailability (3.79±2.68%) comparable to that of DON (5.56±2.05%). After PO DON3G administration to pigs only DON was recovered, indicating a complete presystemic hydrolysis of DON3G. However, the absorbed fraction is approximately 5 times lower than after PO DON administration, 16.1±5.4% compared to 81.3±17.4%. Analysis of phase II metabolites revealed that DON biotransformation in pigs consists mainly of glucuronidation, and in chickens predominantly sulfation occurs, which is absent in pigs. Phase II biotransformation is much more extensive for chickens than for pigs, which is a possible explanation for the differences in sensitivity of the latter species to DON. Furthermore, given the *in vivo* hydrolysis of DON3G in pigs, pigs are likely to be more sensitive to this modified mycotoxin compared to chickens. Although *in vitro* studies reported that DON3G is less toxic than DON, the demonstrated *in vivo* hydrolysis to DON in pigs, as well as the inter-species differences observed, indicate a decreased yet not neglectable toxicological relevance of DON3G and consequently warrant further research to establish a tolerable daily intake.

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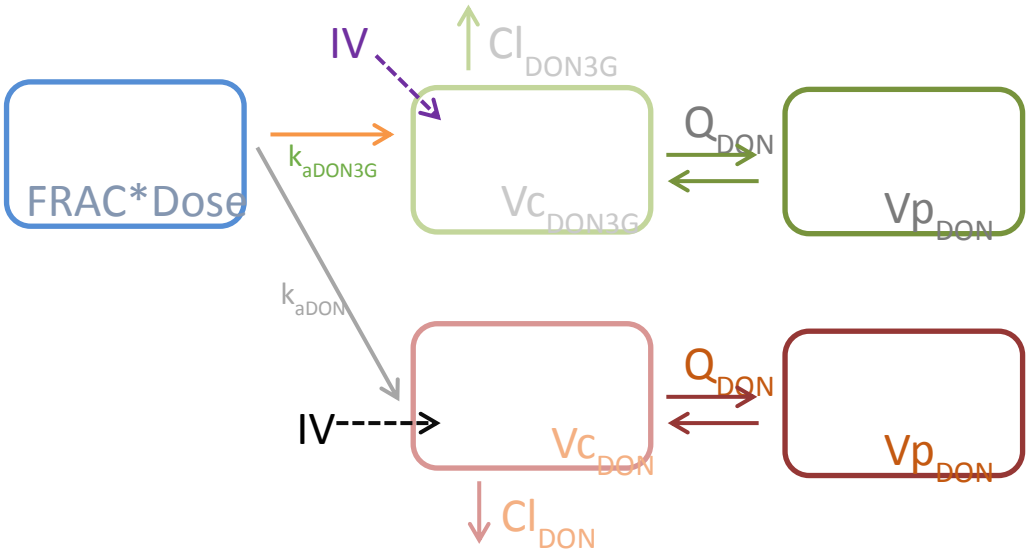

Figure 1. *Graphical representation of the applied model for broiler chicken: DON IV (black, light red and dark red section), DON PO (blue, grey, light red and dark red section), DON3G IV (purple, light green and dark green section) and DON3G PO (blue, orange, light green and dark green section), IV=intravenous administration; ka_{DON}=absorption rate constant of DON (1/min); ka_{DON3G}=absorption rate constant of DON3G (1/min); Cl_{DON}=clearance of DON (mL/min/kg); Q_{DON}=intercompartment flow for DON (mL/min/kg); Vc_{DON}=central volume of distribution for DON (mL/kg); Vp_{DON}=peripheral volume of distribution for DON (mL/kg); Cl_{DON3G}=clearance of DON3G (mL/min/kg); Q_{DON3G}=intercompartment flow for DON3G (mL/min/kg); Vc_{DON3G}=central volume of distribution for DON3G (mL/kg); Vp_{DON3G}=peripheral volume of distribution for DON3G (mL/kg); FRAC=fraction of dose absorbed.*

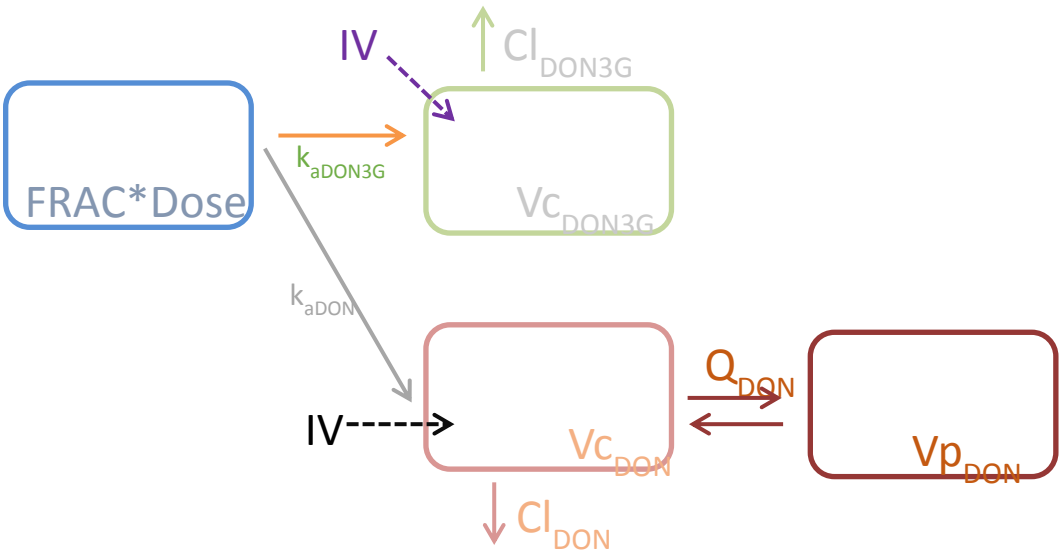

Figure 2. *Graphical representation of the applied model for pig: DON IV (black, light red and dark red section), DON PO (blue, grey, light red and dark red section), DON3G IV (purple and light green section) and DON3G PO (blue, grey and light red section + lag time). IV=intravenous administration; ka_{DON}=absorption rate constant of DON (1/min); ka_{DON3G}=absorption rate constant of DON3G (1/min); Cl_{DON}=clearance of DON (mL/min/kg); Q_{DON}=intercompartment flow for DON (mL/min/kg); Vc_{DON}=central volume of distribution for DON (mL/kg); Vp_{DON}=peripheral volume of distribution for DON (mL/kg); Cl_{DON3G}=clearance of DON3G (mL/min/kg); Vc_{DON3G}=central volume of distribution for DON3G (mL/kg); FRAC=fraction of dose absorbed.*

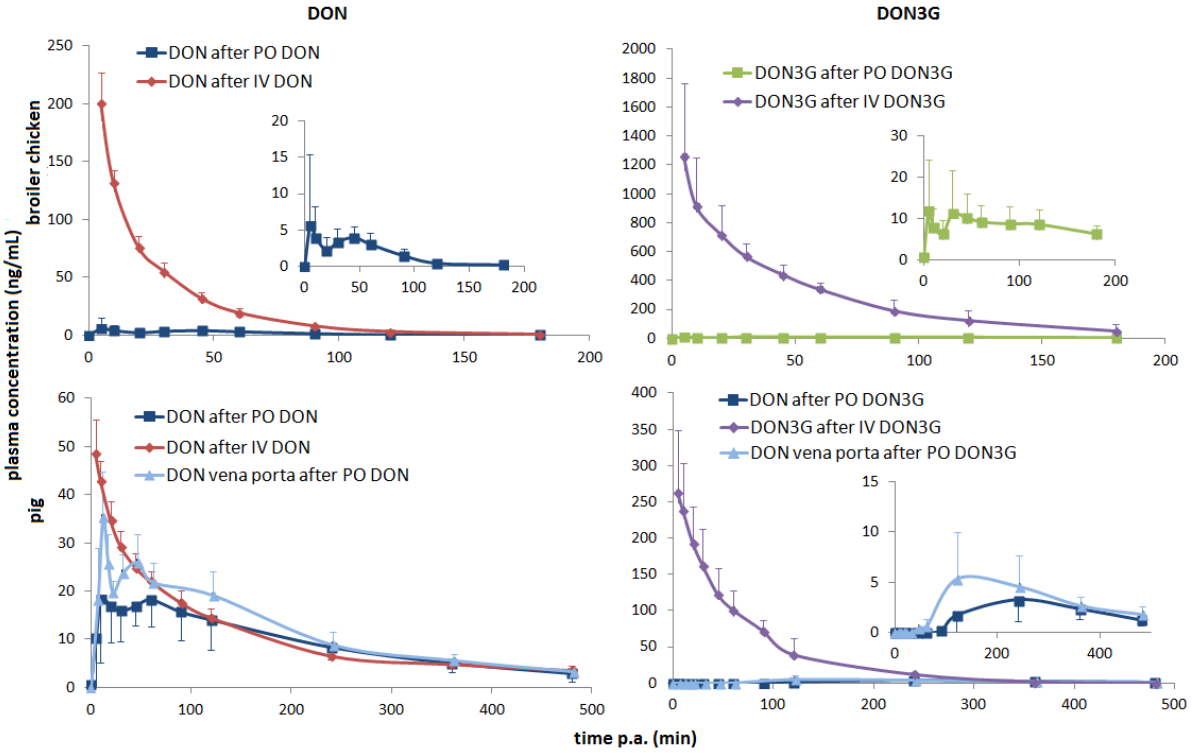

Figure 3. *Systemic plasma (chickens and pigs) and vena porta plasma (pigs) concentration-time profiles of deoxynivalenol (DON) and deoxynivalenol-3-β-D-glucoside (DON3G) after oral (PO) and intravenous (IV) administration (post administration=p.a.) of DON (dose=500 µg DON/kg body weight) and DON3G (dose=774 µg DON3G/kg body weight) to broiler chickens and of DON (dose=36 µg DON/kg body weight) and DON3G (dose=55.7 µg DON3G/kg body weight) to pigs (n=6 for systemic plasma, n=4 for plasma from vena porta). Values are presented as mean + or - SD. No DON was detected in chickens after DON3G administration. In pigs, no DON was detected after IV DON3G administration and no DON3G after PO DON3G administration. The inserts show PO administration data in more detail.*

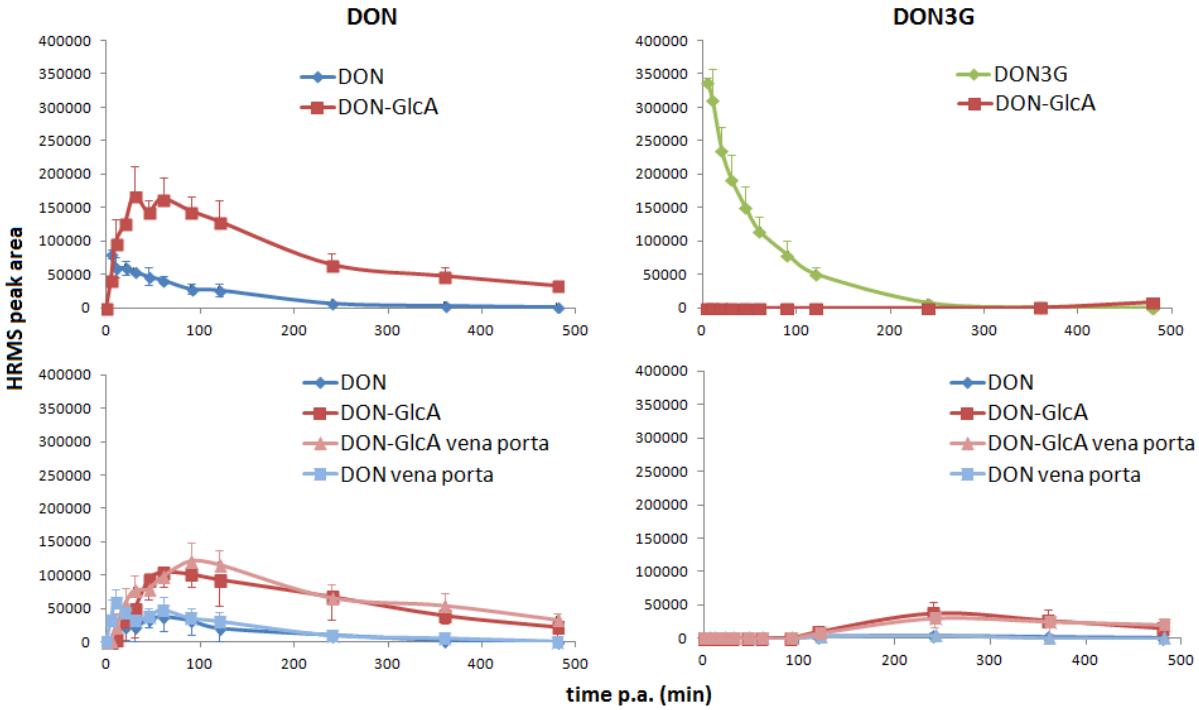

Figure 4. *Porcine plasma (systemic and vena porta) concentration-time profiles of deoxynivalenol (DON), deoxynivalenol-3-β-D-glucoside (DON3G) and deoxynivalenol-glucuronide (DON-GlcA) after oral (PO) and intravenous (IV) administration of DON (dose=36 µg DON/kg body weight) and DON3G (dose=55.7 µg DON3G/kg body weight) to pigs (n=3 for systemic plasma, n=3 for plasma from vena porta). Values are presented as mean + or - SD.*

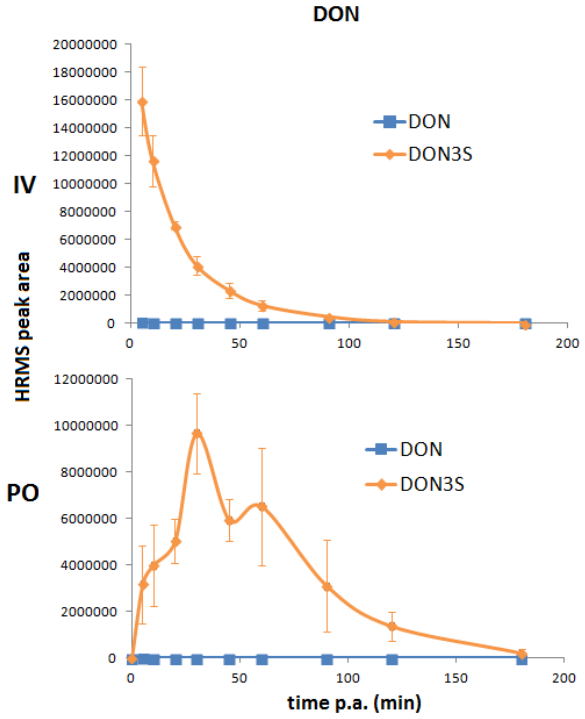

Figure 5. *Plasma concentration-time profiles of deoxynivalenol (DON) and deoxynivalenol-3-sulfate (DON3S) after oral (PO) and intravenous (IV) administration of DON (dose=500 µg DON/kg body weight) to broiler chickens (n=3). Values are presented as mean + SD.*

Table 1. *Compound specific MRM ion transitions and MS-parameters for the analysis of DON, DON3G, DOM-1 and $^{13}C_{15}$-DON in broiler and pig plasma; Rt= retention time; IS= internal standard; [a] quantifier ion.*

	Precursor ion (m/z)	Product ion (m/z)	Rt (min)	Cone voltage (V)	Collision Energy (eV)	Dwell time (ms)	ESI modus
DON	297.1 $[M+H]^+$	249.1[a] 203.4	2.5	20	9 14	0.25 0.25	+
DON3G	517.0 $[M+CH_3COO]^-$	427.2[a] 247.0	2.8	14	16 15	0.25 0.25	-
DOM-1	281.1 $[M+H]^+$	215.1[a] 137.0	4.0	20	12 16	0.81 0.81	+
$^{13}C_{15}$-DON (IS)	312.0 $[M+H]^+$	245.2[a] 263.0	2.5	20	10 10	0.25 0.25	+

Table 2. *Validation results for linearity (r & gof, 8 concentration points in the range LOQ-500 ng/mL), sensitivity (LOD & LOQ; n=6), matrix effects (SSE), apparent recovery (R_A) and extraction recovery (R_E).*

Plasma	Compound	Correlation coefficient (r)	Goodness of fit (gof)	LOD (ng/mL)	LOQ (ng/mL)	SSE (%)	R_A (%)	R_E (%)
Chicken	DON	0.9998	4.92%	0.47	1.0	85.3	65.9	77.2
	DOM-1	0.9997	7.05%	0.11	1.0	112.0	76.2	68.2
	DON3G	0.9997	7.58%	0.38	1.0	58.1	43.9	75.6
Pig	DON	0.9997	5.94%	0.01	0.5	67.5	65.4	97.0
	DOM-1	0.9999	3.98%	0.28	1.0	44.0	41.8	95.0
	DON3G	0.9998	4.98%	0.03	0.1	73.1	64.8	88.7

Table 3. *Validation results for within-day (n=6) and between-day precision (n=3x3) with corresponding accuracy at low (LOQ) and high (500 ng/mL) concentration level.*

		Within-day				Between-day			
		Accuracy (%)		Precision (RSD, %)		Accuracy (%)		Precision (RSD, %)	
Plasma	Compound	LOQ	500 ng/mL	LOQ	500 ng/mL	LOQ	500 ng/mL	LOQ	500 ng/mL
Chicken	DON	8.0	0.6	12.3	3.3	-9.5	-0.7	27.6	4.7
	DOM-1	15.6	0.7	24.1	4.6	-12.2	0.3	12.7	4.2
	DON3G	6.1	0.3	29.4	7.5	16.3	0.3	18.4	5.0
Pig	DON	-1.3	0.8	31.4	3.3	-5.7	-0.8	28.8	3.6
	DOM-1	-7.7	-0.6	6.4	2.0	-2.3	1.0	18.2	3.4
	DON3G	-11.4	-0.8	24.6	1.7	-1.1	-2.0	31.3	4.3

Table 4. *Toxicokinetic parameters, presystemic hydrolysis of the bioavailable fraction (%) and absolute oral bioavailability (F%) of DON and DON3G after IV and PO administration to broiler chickens and pigs (n=6).*

	Broiler chicken	Pig

	DON (IV&PO)	DON3G (IV&PO)	DON (IV&PO)	DON3G IV	DON3G PO
ka_{DON} (1/min)	0.0315±0.0173	-	0.0274±0.0107	-	0.00501±0.00160
Ke_{DON}/β_{DON} (1/min)	0.0369±0.0041	-	0.00425±0.00155	-	0.0217±0.0014
$t_{1/2elDON}$ (min)	18.8±2.1	-	163±59	-	31.9±2.1
Cl_{DON} (mL/min/kg)	88.8±12.4	-	5.25±1.25	-	25.9±2.8
Q_{DON} (mL/min/kg)	86.7±40.7	-	17.4±11.3	-	-
Vc_{DON} (mL/kg)	1350±578	-	614±127	-	1196±717
Vp_{DON} (mL/kg)	1087±211	-	789±486	-	-
t_{lag} (min)	-	-	-	-	83.6±5.6
ka_{DON3G} (1/min)	-	0.0238±0.0203	-	-	-
ke_{DON3G}/β_{DON3G} (1/min)	-	0.0204±0.0037	-	0.0128± 0.0007	-
$t_{1/2elDON3G}$ (min)	-	34.0±6.2	-	54.1±3.0	-
Cl_{DON3G} (mL/min/kg)	-	11.4±1.2	-	3.17±0.81	-
Q_{DON3G} (mL/min/kg)	-	27.7±25.8	-	-	-
Vc_{DON3G} (mL/kg)	-	290±188	-	249±67	-
Vp_{DON3G} (mL/kg)	-	284±167	-	-	-
FRAC (%)	5.56±2.05	3.79±2.60	81.3±17.4	-	16.1±5.4**
Pres.Hydr. (%)	-	0	-	-	100
F (%)	5.56±2.05	3.79±2.68	81.3±17.4	-	0*
C_{max} (ng/mL)	7.40±7.35	16.6±10.4	23.7±9.8	-	3.88±2.0**
t_{max} (min)	30.0±22.5	43.1±49.4	44.4±39.1	-	225±100**

*Values are presented as mean ± SD. For pigs, the DON3G PO parameters have been calculated simultaneously on all pooled pig PO DON3G data, as a result no SD could be calculated, instead coefficients of variation of the models' predicted versus the observed concentrations are given. ka_{DON}=absorption rate constant for DON (1/min); ke_{DON}=elimination rate constant for DON (1/min); β_{DON}=disposition rate constant for DON (1/min); $t_{1/2elDON}$=elimination half-life of DON (min); Cl_{DON}=clearance of DON (mL/min/kg); Q_{DON}=intercompartmental flow for DON (mL/min/kg); Vc_{DON}=central volume of distribution for DON (mL/kg); Vp_{DON}=peripheral volume of distribution for DON (mL/kg); t_{lag}=absorption lag time (min); ka_{DON3G}=absorption rate constant of DON3G (1/min); ke_{DON3G}=elimination rate constant for DON3G (1/min); β_{DON3G}=disposition rate constant for DON3G (1/min); $t_{1/2elDON3G}$=elimination half-life of DON3G (min); Cl_{DON3G}=clearance of DON3G (mL/min/kg); Q_{DON3G}=intercompartmental flow for DON3G (mL/min/kg); Vc_{DON3G}=central volume of distribution for DON3G (mL/kg); Vp_{DON3G}=peripheral volume of distribution for DON3G (mL/kg); FRAC=absorbed fraction; Pres.Hydr.=percentage of the total dose that is hydrolysed presystemically to DON and absorbed as DON (%); F=absolute oral bioavailability (%); C_{max}=maximum plasma concentration for DON (ng/mL); t_{max}= time to maximum plasma concentration for DON (min); * and ** statistically significant difference at $p<0.05$ and $p<0.01$, respectively, compared to DON values in the same animal species.*