

The fate and tissue disposition of deoxynivalenol in broiler chickens

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ABSTRACT. To evaluate the fate of deoxynivalenol (DON) in broilers, DON was administered either intravenously or orally to broilers at a dose of 1 mg/kg BW. Concentrations of DON in plasma were measurable up to 4 hr and 2 hr after intravenous and oral administration, respectively. Following intravenous administration, the values for the elimination half-life, the volume of distribution and the clearance were 1.25 ± 0.25 hr, 7.55 ± 2.03 l/kg and 4.16 ± 0.42 l/hr/kg, respectively. The oral bioavailability was $15.46 \pm 4.02\%$. DON was detectable in all tissues examined after oral administration. These results suggest that DON is able to penetrate into the various tissues in broilers, though poorly absorbed from their gastrointestinal tract.

KEY WORDS: broiler, deoxynivalenol, fate residue, residue broilers

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Deoxynivalenol (DON, vomitoxin) is a most commonly occurring trichothecene mycotoxin produced by several plant pathogenic fungi, of which *Fusarium graminearum* and *Fusarium culmorum* are the most important sources. DON contamination has been found in a variety of food-stuffs worldwide, including wheat, maize, barley, oats, rice and feed for farm animals [5, 10–12, 33] (<http://www.efsa.europa.eu/de/efsajournal/doc/73.pdf>; http://www.who.int/foodsafety/chem/summary72_rev.pdf) and also in cereals in South America, Canada, European Union countries, etc. [14]. The contamination has accompanied with various adverse health effects, such as feed refusal, anorexia, weight loss, vomiting and immunotoxicity in animals [7, 8, 23, 25, 30]. Consistent with this, DON is stable during the storage and processing of agricultural products [27, 29]. Toxic effects of DON on farm animals have repeatedly been reviewed with the anorectic and immune-modulatory effects being most pronounced in pigs [4, 5] (<http://www.efsa.europa.eu/de/efsajournal/doc/73.pdf>). Human food poisoning after ingestion of DON has also been reported. The symptoms described include abdominal pain or a feeling of fullness in the abdomen, dizziness, headache, throat irritation, nausea, vomiting, diarrhea and blood in stools [28]. DON suppresses normal immune response to pathogens and simultaneously induces autoimmune-like effects, which are similar to human immunoglobulin A (IgA) nephropathy [2, 16, 24].

The toxicokinetic characteristics and tissue residues of

DON have been studied in livestock and mice, but limited information is available on the fate and tissue disposition of DON in broilers. We studied therefore the fate and tissue residues of DON in broiler chickens by determining toxicokinetic parameters and toxin depletion in various tissues.

DON purchased from Wako Chemical Co. (Tokyo, Japan) was dissolved in 0.9% physiologic saline to the final concentration of 2 mg/ml for administration. Other reagents and chemicals of an analytical grade were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Purified water was produced using the Milli-Q water purification system from Millipore, Inc. (Bedford, MA, U.S.A.).

Three-week-old female broiler chickens (average body weight, 1.24 ± 0.21 kg) were obtained from a chicken farm (CP Group Co.), Saraburi Province, Thailand, and acclimatized for 1 week. Each bird was placed in individual stainless-steel cages at the Laboratory Animal Facility, Faculty of Veterinary Medicine, Kasetsart University, with water and feed available *ad libitum*. All experimental procedures were ethically approved by Kasetsart University Animal Care and Use Committee.

In the first experiment, toxicokinetic characteristics of DON were investigated using ten broilers divided into 2 groups (n=5). After overnight fasting, each group was administered intravenously (i.v.) or orally (p.o.) with DON at a dose of 1.0 mg/kg BW. This dose level was selected based on previous studies by Anonymus [1] and Poapolathep *et al.* [17]. Following i.v. or p.o. administration of DON, there were no any adverse effects in chickens. 2.5 ml blood samples was collected from the wing veins of each animal with heparinized syringes at 0, 5, 15 and 30 min and at 1, 2, 4, 8 and 12 hr after DON administration. Plasma was separated by centrifugation ($1,968 \times g$) for 15 min.

In the second experiment, tissue residues of DON were investigated using 25 broilers. Twenty of them were orally administered with DON at a dose of 1 mg/kg of BW, and

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5 were orally administered with 0.9% physiologic saline as a control. They were sacrificed by i.v. administration of thiopentone sodium at a dose of 20 mg/kg BW. Tissue samples, including livers, kidneys, muscle and small intestines, were taken from 5 birds each at 1, 3, 6 or 12 hr after administration. Excreta were collected at 0, 1, 3, 6 and 12 hr after administration. All samples were frozen at -20°C until analysis.

DON was extracted from blood plasma as described previously with some modifications [17, 32]. Briefly, 1 ml of plasma diluted with 0.5 ml of 0.01 M phosphate buffer (PB, pH 6.8) was applied on a ChemElut column (Varian Inc., Palo Alto, CA, U.S.A.), which was then eluted with 50 ml of ethyl acetate. The eluate was evaporated to dryness under vacuum at 40°C , dissolved in 2 ml of 5% polyethylene glycol (PEG) and filtrated with a Minisart[®] RC filter (pore size: $0.45\ \mu\text{m}$, Sartorius AG, Goettingen, Germany). The filtrate was applied on an immunoaffinity column (IAC, DONPrep[®], R-Biopharm Rhone Ltd., Darmstadt, Germany). After the column was washed twice with 2.5 ml of water and flushed with air, DON was eluted with 3 ml of methanol. The eluate was then evaporated to dryness under a nitrogen stream at 50°C on a heating block. The residue was reconstituted with 1 ml of methanol/water (10/90, v/v). After being passed through a Minisart[®] RC filter (pore size: $0.45\ \mu\text{m}$, Sartorius AG), the reconstituted residue was analyzed by liquid chromatography/tandem mass spectrometry (LC/MS/MS).

Preparation of tissue samples for DON analyses was performed according to the method described previously, with some modifications [22, 31]. Tissues (4 g of each tissue sample) were homogenized in 8 ml of water (Milli-Q) with an Ultra-Turrax homogenizer (IKA Works, Inc., Wilmington, DE, U.S.A.). The homogenates were mixed with 16 ml of acetonitrile (ACN), and the supernatant was separated by centrifugation at $1,968 \times g$ for 15 min. Aliquot samples of 5.6 ml per each tissue sample were passed through a charcoal alumina column (Trilogy Analytical Laboratory, Washington, MO, U.S.A.). DON was eluted with 10 ml of ACN/water (21/4, v/v) and evaporated to dryness under a nitrogen stream at 50°C . The sample was subjected to LC/MS/MS.

The LC analysis was performed using an Agilent 1200 series system consisting of a binary high-pressure gradient pump, a vacuum solvent degassing unit, an automatic sample injector and a column thermostat (Agilent Technologies, Waldbronn, Germany) equipped with a Poroshell 120 EC-C18 column ($2.7\ \mu\text{m}$, $3.0 \times 50\ \text{mm}$) (Agilent Technologies, Palo Alto, CA, U.S.A.) maintained at 40°C . The LC mobile phase program consisted of a binary gradient of the 5 mM ammonium acetate with 0.1% acetic acid in water (mobile phase A) and the 5 mM ammonium acetate with 0.1% acetic acid in methanol (mobile phase B). The composition started out at 5% mobile phase B and increased linearly to 70% mobile phase B by 7 min. The mobile phase then returned to 5% methanol by 8 min, and the column was equilibrated for 2 min. The flow rate was $250\ \mu\text{l}/\text{min}$, the injection volume was $5\ \mu\text{l}$, and the needle was flushed with $250\ \mu\text{l}$ of 100% methanol between samples.

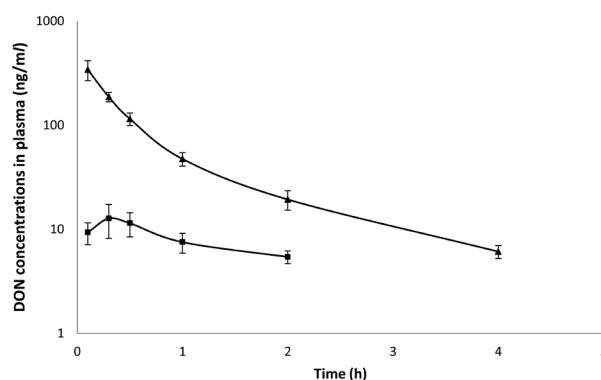


Fig. 1. Mean values (\pm SD) of deoxynivalenol concentrations in plasma broiler chickens at a dosage of 1 mg/kg BW; (▲) intravenous administration, (■) oral administration ($n=5$).

Mass spectrometry was performed using an Agilent Technologies 6460 triple guard mass spectrometer equipped with an electrospray ionization (ESI) source and Agilent MassHunter Workstation Software version 1.2. ESI-MS/MS was operated at unit mass resolution in multiple reaction monitoring (MRM) negative ion mode with the following settings: nebulizer gas pressure (NEB): 45 psi, gas flow $5.0\ \text{l}/\text{min}$, gas temperature 300°C and Capillary voltage: -3500V . The molecular ions and fragments were as follows: Q1: m/z 355 $[\text{M}-\text{H}]^{-}$, Q3 (1): m/z 265, CE (1): 5 eV, Q3 (2): m/z 59.1 and CE (2): 9 eV.

To evaluate recovery, DON was added to samples of blank plasma and tissues to yield final DON concentrations of 1, 5, 10, 50, 100 and $500\ \text{ng}/\text{ml}$ (ng/g) and analyzed in duplicate. The mean (\pm SD) recoveries of DON were $91.16 \pm 4.23\%$, $83.62 \pm 2.14\%$, $84.67 \pm 5.18\%$, $76.36 \pm 3.15\%$, $83.11 \pm 2.66\%$ and $73.69 \pm 3.06\%$ in the plasma, liver, kidney, muscle, small intestine and excreta, respectively. The limit of detection (LOD) of DON was $1\ \text{ng}/\text{ml}$ (ng/g). The r^2 value of the DON calibration curves was 0.996, and the precision and accuracy indicated the method to be repeatable. The intra- and inter-day precisions were $<11\%$.

The concentration of DON in plasma was pharmacokinetically analyzed using a two-compartment model with the PK Solutions 2.0TM Program (Summit Research Services, Montrose, CO, U.S.A.) including K_{el} (elimination rate constant), K_{12} , K_{21} (micro-rate constants), $AUC_{0-\infty}$ (area under the curve), $t_{1/2\beta}$ (elimination half-life), $t_{1/2\alpha}$ (distribution half-life), V_d (area) (volume of distribution), Cl (clearance) and MRT (mean residence time). The absolute oral bioavailability ($F_{p.o.}$) was calculated using the following equation: $(\%)F = (AUC_{p.o.})/(AUC_{i.v.}) \times 100$.

DON was detectable in plasma following a single i.v. or p.o. administration. The semi-logarithmic plots of the mean (\pm SD) plasma concentration-time curves of DON are shown in Fig. 1. DON was detectable from 5 min to 4 hr after i.v. administration and from 5 min to 2 hr after p.o. administration. The plasma profile displayed a rapid decrease in the DON concentration with time in both the groups. The data were shown to best fit a two-compartment model. As shown

Table 1. Mean \pm SD value of the toxicokinetic parameters of deoxynivalenol following a single intravenous or oral administration at a dosage of 1 mg/kg BW in broiler chickens (n=5)

Toxicokinetic parameters (unit)	i.v.	p.o.	P-value
K_{el} (hr)	0.57 ± 0.098	0.32 ± 0.17	0.02
K_{12} (hr ⁻¹)	1.14 ± 0.57	-	-
K_{21} (hr ⁻¹)	0.92 ± 0.22	-	-
$t_{1/2\alpha}$ (hr)	0.21 ± 0.10	0.34 ± 0.12	0.09
$t_{1/2\beta}$ (hr)	1.25 ± 0.25	2.69 ± 0.96	0.01
CL (l/hr/kg)	4.16 ± 0.42	2.83 ± 0.74	0.01
V_d (l/kg)	7.55 ± 2.03	10.04 ± 3.09	0.17
MRT (hr)	2.10 ± 0.54	3.92 ± 1.76	0.06
C_{max} (ng/ml)	-	12.73 ± 4.06	-
T_{max} (min)	-	15.0 ± 0.0	-
$F_{p.o.}$ (%)	-	15.46 ± 4.02	-

K_{el} =elimination rate constant; K_{12} , K_{21} =micro-rate constants. $t_{1/2\alpha}$ =distribution half-life; $t_{1/2\beta}$ =elimination half-life; CL=clearance. V_d =Volume of distribution; MRT=mean residence time, C_{max} =maximum concentration. T_{max} =time at maximum concentration; $F_{p.o.}$ =oral bioavailability. A statistical difference was calculated using the Student's *t*-test; $P < 0.05$ was judged to be significant; $P < 0.01$ was judged to be highly significant.

Table 2. Mean \pm SD residue concentrations of deoxynivalenol (DON) in various tissues following a single oral administration at 1 mg/kg BW in broiler chickens (n=5)

Time (hr)	DON concentration (ng/g)				
	Liver	Kidney	Small intestine	Muscle	Excreta
1	5.58 ± 0.23	6.18 ± 2.74	57.05 ± 10.95	4.25 ± 1.56	94.49 ± 12.42
3	2.23 ± 0.33	2.35 ± 0.75	2.55 ± 0.08	ND	74.58 ± 7.77
6	ND	ND	ND	ND	27.93 ± 0.90
12	ND	ND	ND	ND	37.34 ± 6.78

ND=not detected

in Table 1, the values for the $t_{1/2\beta}$, V_d , CL and MRT in iv treated birds were 1.25 ± 0.25 hr, 7.55 ± 2.03 l/kg, 4.16 ± 0.42 l/hr/kg and 2.10 ± 0.54 hr, respectively. The maximum plasma concentration (C_{max}) of DON was 12.73 ± 4.56 ng/ml at 15 min after p.o. administration. The absorption of DON from gastrointestinal tract was low with an average absolute oral bioavailability of 15.46%. DON was also detected in excreta up to 12 hr following p.o. administration. The LC-MS/MS profile for various tissues showed that DON was measurable up to 3 hr in livers, kidneys and small intestines, whereas it was detectable up to 1 hr in muscle after p.o. administration (Table 2). The maximum levels of DON in the liver, kidney, small intestine and muscle were 2.58 ± 0.23 ng/g, 6.18 ± 2.74 ng/g, 57.05 ± 10.95 ng/g and 4.25 ± 1.56 ng/g, respectively, at 1 hr after p.o. administration.

DON is a major contaminant in food and feedstuffs. Our study was designed to characterize the fate and tissue residues of DON after i.v. or p.o. administration at a dose of 1 mg/kg BW in broiler chickens. De-epoxydeoxynivalenol (DOM-1), a major metabolite of DON formed in animals, was not investigated, because Prelusky *et al.* [21] reported that 95% was presented as the parent toxin, metabolic conversion to DON conjugate was estimated at less than 5%, and the

nontoxic DOM-1 was not detected after oral administration of DON in animals. A previously reported pharmacokinetic study described DON in plasma of pigs, broilers and sheep [3, 9, 15, 18, 19, 21]. Following i.v. administration, the $t_{1/2\beta}$ indicates the overall rate of elimination and allows the prediction of DON accumulation with a value for DON of 1.25 hr in broiler chickens. The mean residence time (MRT, the average time that DON molecule resides in the body) was 2.10 hr after i.v. administration. DON appears to have been excreted rapidly in broiler chickens. Thus, about 99% of the DON from broiler plasma was cleared within 9 hr after i.v. administration. The $t_{1/2\beta}$ of DON obtained in this study was longer than that reported (30 min) earlier in broilers [13]. However, that study did not determine the residue of DON in edible tissues of broilers. Furthermore, the $t_{1/2\beta}$ of DON in the present study was shorter than that reported in growing pigs (2.1–8.8 hr) [17, 21], whereas it was similar in sheep (1.1–1.3 hr) [18, 20]. The V_d of DON in broiler chickens was 7.55 l/kg after i.v. administration. These results suggest that DON might have penetrated to various tissues, although it appears to be excreted rapidly after i.v. administration in broiler chickens.

Following p.o. administration, the C_{max} was 12.73 ng/ml,

and the absolute oral bioavailability of DON was 15% in broiler chickens in this study. Prelusky *et al.* [19] reported a rapid transport of DON-derived radioactivity through the alimentary tract of broiler chickens, indicating the poor absorption of DON. The $F_{p.o.}$ of DON in broiler chickens of the present study was lower than that in pigs (48–110%) [9, 21], but it was higher than that in sheep [18]. The $F_{p.o.}$ of DON in the present study corresponded well to the previous findings in broiler chickens (19%) [15]. The level of DON in various tissues, including the liver, kidney and small intestine, was detectable up to 3 hr, whereas it was measurable up to 1 hr in muscle after p.o. administration of DON in broiler chickens. The tissue level of DON was high in the small intestine, kidney, liver and muscle, in this order. This also indicates the ability of DON to penetrate into various tissues of broiler chickens, although the toxicokinetic study revealed a low absolute oral bioavailability. Taken together, it becomes clear that the small intestine, kidney, liver and muscle of broiler chickens could contribute to the human exposure to DON.

The lowest and highest tissue concentrations of DON were found in muscle (4 ng/g) and in small intestine (57 ng/g), respectively. Regarding the acute and chronic threshold of 8 µg/kg BW per day [11] (http://www.who.int/foodsafety/chem/summary72_rev.pdf) and 1 µg/kg BW per day [26] (http://ec.europa.eu/food/fs/sc/scf/out123_en.pdf), it is unlikely therefore that DON will cause harm to humans whose average meat consumption was 42.9 kg/person/year or average of 117 g/day [6] (<http://www.fao.org/3/a-i4136e.pdf>). The results obtained in this study supplemented those of our previous study may contribute to our understanding of toxicokinetic characteristics and residues of mycotoxins, particularly DON, in food producing animals.

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