JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Cite This: J. Agric. Food Chem. 2018, 66, 2129−2135

pubs.acs.org/JAFC

Comparative Toxicokinetics and Plasma Protein Binding of Ochratoxin A in Four Avian Species

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Supporting Information

ABSTRACT: Ochratoxin A (OTA, 0.25 mg/kg body weight) was absorbed rapidly (T_{max} = 0.31−1.88 h) in all avian species (broiler chickens, laying hens, turkeys, and Muscovy ducks) but more slowly in broiler chickens (T_{max} = 1.43−4.63 h). The absolute oral bioavailability was complete in these bird species (88.0−109.6%). Ducks have a significantly higher volume of distribution (V_d) and turkeys a lower V_d compared to chickens and layers (broiler chickens, 0.27 ± 0.12 L/kg; layers, 0.23 ± 0.08 L/kg; turkeys, 0.18 ± 0.04 L/kg; ducks, 0.76 ± 0.44 L/kg). This difference in V_d can be attributed to the species-dependent differences in plasma protein binding of OTA, namely ranging between 82.2 and 88.9% in ducks and between 96.5 and 98.8% in turkeys. No significant gender differences were found in toxicokinetics or plasma protein binding.

KEYWORDS: *ochratoxin A, toxicokinetics, plasma protein binding, turkey, broiler chicken, layer, duck, birds*

INTRODUCTION

The mycotoxin ochratoxin A (OTA) is a highly prevalent contaminant of feedstuffs, with contamination rates in North America and Central, South, and Eastern Europe of 20%, 29%, 46%, and 49%, respectively.[1] In the latter, 7495 samples were investigated, and the average contamination level of the positive samples was 14 μg/kg feed, ranging up to 1589 μg/kg. OTA has different modes of actions to disrupt cellular physiology.[2] The main effect is with inhibition of the phenylalanine tRNA-complex, therefore disturbing phenylalanine metabolism. Furthermore, it stimulates lipid peroxidation and consequently cellular damage.[3] Besides, this mycotoxin is IARC class 2B classified (carcinogenic in laboratory animals and possibly carcinogenic in humans),[4] despite the fact that the action mechanism has not been well described yet.[5] Molecular targets are histone acetyltransferases (HATs), which are critical in the regulation of a wide range of cellular processes, including DNA damage repair and mitosis.[6,7]

Species-dependent sensitivity toward acute OTA toxicity has been demonstrated.[8] Pigs, for instance, are prone to OTA due to distinct toxicokinetic characteristics including long plasma elimination half-life and kidney tissue accumulation. This can be attributed to high plasma protein binding (99.9%) and enterohepatic and renal recirculation.[8] OTA can cause glucosuria, enzymuria, and a decrease in the transport of *para*-aminohippuric acid (PAH) by disrupting proximal tubular functions.[9] OTA is deemed to be a substrate of the renal organic anion transporters (OATs), which play an important role in OTA accumulation and, consequently, nephrotoxicity.[9] In general, birds excrete OTA faster than mammals, leading to more limited accumulation. More specifically, the elimination half-life of OTA in broiler chickens is significantly shorter than that in pigs (4.1 h versus 150 h), leading to a lower systemic exposure of OTA in chickens.[10]

Although poultry are generally less prone to the effects of OTA, differences in sensitivity between avian species are present. Reported LD_{50} values for birds are 0.5, 3.3, 5.9, and 16.5 mg/kg body weight (BW) for ducks, broiler chickens, turkeys, and Japanese quail, respectively.[11,12] These values point toward a higher sensitivity in ducks compared to turkeys and Japanese quail. Turkeys on the other hand seem to be more susceptible to several other mycotoxins compared to ducks, broiler chickens, or laying hens, such as aflatoxin B1, deoxynivalenol, zearalenone, and fumonisin B1, and these differences can be attributed to differences in absorption, distribution, metabolism, or excretion (ADME) of the mycotoxin.[13−16] Regarding gender-specific toxicity, Zepnik et al.[17] demonstrated a higher accumulation of OTA in kidney tissues of male compared to female F344 rats. Furthermore, gender differences in relative OTA plasma protein binding were observed in the rat. OTA has a three-times higher affinity for plasma proteins in male rats compared to female rats.[18] Both factors might explain the gender related differences in toxicity in rats.

To our knowledge, no comparative ADME and plasma protein binding studies for OTA have been reported in poultry species. Only Galtier et al.[19] have previously studied the toxicokinetics in

Received: December 23, 2017
Revised: February 6, 2018
Accepted: February 8, 2018
Published: February 8, 2018

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birds, but only broiler chickens were used, and no possible gender effects were evaluated. Therefore, the aim was to elucidate the absolute oral bioavailability and disposition of OTA in four avian species (turkeys, broiler chickens, layers, and ducks). Furthermore, differences in clearance of OTA between species may be mainly governed by differences in plasma protein binding. Consequently, plasma protein binding of OTA was assessed in theses avian species as well.

■ MATERIALS AND METHODS

Chemicals, Products, and Reagents. The analytical standard of OTA (animal and analytical experiments) was obtained from Fermentek (Jerusalem, Israel) (storage temperature ≤ −15 °C). Internal standard (IS), $^{13}C_{20}$-OTA (10 μg/mL in acetonitrile (ACN)), used for the analytical experiments was obtained from Romer Laboratories (Tulln, Austria) (storage temperature ≤ −15 °C) . A stock solution of OTA of 1 mg/mL was prepared in ACN. Working solutions of OTA and the IS for the analytical experiments were prepared by appropriate dilution of the stock solutions with ACN (storage temperature: 2−8 °C). Water, methanol (MeOH), ACN, ammonium acetate, formic acid (FA), and glacial acetic acid used for the plasma analysis were of LC−MS grade and were obtained from Biosolve (Valkenswaard, The Netherlands). Water and ethanol used for the animal experiment were obtained from Filterservice (Eupen, Belgium). Oasis Ostro protein precipitation and phospholipid removal 96-well plates (25 mg) (Waters, Zellik, Belgium) were used for sample preparation.. Amicon Ultra (Ultracel 30 kDa, regenerated cellulose) Centrifugal Filter Units (Merck-Millipore (Overijse, Belgium) were used for the determination of OTA plasma protein binding were obtained from.

Animals and Experimental Procedure. Eight turkeys (Hybrid Converter poults, 4♂/4♀, 1.71 ± 0.14 kg BW, 5 weeks old), broiler chickens (Ross 308, 4♂/4♀, 1.27 ± 0.10 kg BW, 4 weeks old), layers (Lohmann Brown-Lite, 4♂/4♀, 2.05 ± 0.35 kg BW, 20 weeks old), and Muscovy ducks (4♂/4♀, 2.68 ± 0.63 kg BW, 6 months old) were all obtained from commercial farms. Animals were housed in group per species, the light cycle was 16 h light/8 h dark. Ad libitum feed and water were available during the acclimatization period (1 week). The administered feed for turkeys, broiler chickens, layers, and ducks was Dindo 2.1 pellet, Farm pellet 2, Golf 4 pellet, and Duck 3 pellet, respectively (Versele-Laga, Deinze, Belgium). Mycotoxin contamination was evaluated in the feed according to Monbaliu et al.,[20] and no OTA could be detected (Supplementary Table 1). The ducks had access to a pool of 1 m^3.

Subsequently, 8 h before experiment initiation, animals were fasted. Next, four animals (2♂/2♀) per species were given OTA (0.25 mg/kg BW) orally by gavage (PO), and the other four birds/species were given the same dose of OTA by injection in the wing vein (IV). The PO and IV bolus solution was prepared by dissolving the OTA standard in ethanol (10 mg/mL) and further diluted with water (PO) or physiological saline (IV) up to a volume of 1 mL. After OTA administration, 0.5 mL of blood was collected (leg vein) in heparinized tubes (Terumo Europe, Haasrode, Belgium) at 0 (just before administration) and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 36 h after administration. Samples were centrifuged (2851 × g, 10 min, 4 °C), and plasma aliquots (100 μL) were stored (≤ −15 °C). Animals were given access to feed at 4 h after OTA administration. After a six-day wash-out period, the protocol was repeated, but the birds that received an IV injection of OTA then received OTA orally and vice versa (two-way crossover). Animals and samples were handled in the same manner. Approval of the animal experiments was granted by the Ethical Committee of the Faculties of Bioscience Engineering and Veterinary Medicine (Ghent University) (case no. EC 2014/118).

Analysis of Plasma Samples of Toxicokinetic Study. Plasma sample pretreatment was performed by removing phospholipids and precipitation of proteins using Oasis Ostro plates. A sample of 12.5 μL of the IS working solution (1 μg/mL) was added to plasma (100 μL) as well as 100 μL of ACN/water (50:50, v/v), followed by vortex mixing (15 s). Next, samples were brought onto an Oasis Ostro plate, and 400 μL of 1% FA (in ACN) was added, followed by aspiration (5 times) to mix the samples. Vacuum was applied to the Oasis Ostro 96-well plate for 5 min (25 mmHg), and the filtrate was collected in a 2 mL square collection plate.

The sample extracts were analyzed using the liquid chromatography−tandem mass spectrometry (LC−MS/MS) method as previously described by Devreese et al.[21] Chromatographic separation was performed by a Hypersil Gold column (50 mm × 2.1 mm i.d., dp: 1.9 μm), and the guard column was the same type (10 mm × 2.1 mm i.d., dp: 3 μm) (ThermoFisher Scientific, Breda, The Netherlands). Mobile phase A was 5 mM ammonium acetate in water/methanol/acetic acid (94:5:1, v/v/v), whereas mobile phase B was ACN. Gradient elution was used with a flow rate of 300 μL/min and column oven temperature of 45 °C and autosampler tray temperature of 5 °C. The effluent was coupled to a TSQ Quantum Ultra triple quadrupole mass spectrometer, with a heated electrospray ionization (h-ESI) probe (negative ionization mode) (ThermoFisher Scientific). The following transitions (m/z) were monitored in the multiple reaction monitoring mode (MRM) OTA, 402.2 > 358.2* and 402.0 > 166.6; and for the IS, 422.0 > 377.1* and 422.0 > 175.1. The quantifier transition is denoted with an ∗. The limit of quantification in all avian species (broiler chicken, layer, turkey, and duck) was 5.0 ng/mL.

Plasma Protein Binding Study. Plasma protein binding of OTA was assessed by spiking fresh blank plasma of the different species at 0.5, 1, and 5 μg/mL. At each concentration level, one aliquot (aliquot 1) followed the plasma sample treatment (vide supra). Second and third aliquots (aliquots 2 and 3) were incubated in a hot water bath, set at 41 °C. After 1 h, aliquots 2 and 3 were transferred onto Amicon Ultra centrifugal filter devices. Following centrifugation (8517g, 15 min, 41 °C), 20 μL of the filtrate of aliquots 2 and 3 was transferred to an Eppendorf cup, and 12.5 μL of the IS working solution (1 μg/mL), 100 μL of ACN/water (50:50, v/v), and 80 μL of water were added and vortex mixed. Samples were transferred to an Oasis Ostro 96-well plate and treated further on in a similar way as aliquot 1.

The sample extracts of the plasma protein binding study were analyzed using the following LC−MS/MS method: the UPLC system consisted of an Acquity UPLC H-Class Quaternary Solvent Manager and Flow-Through-Needle Sample Manager with temperature controlled tray and column oven from Waters (Zellik, Belgium). Chromatographic separation was achieved on an Acquity BEH C18 column (50 mm × 2.1 mm i.d., dp: 1.8 μm) in combination with a Vanguard precolumn of the same type, both from Waters. The mobile phase A consisted of 5 mM ammonium acetate in water/methanol/acetic acid (94:5:1, v/v/v), whereas mobile phase B was ACN. A gradient elution was performed: 0−1.0 min (65% A, 35% B), 1.5 min (linear gradient to 90% B), 1.5−3.5 min (10% A, 90% B), 3.7 min (linear gradient to 65% A), 3.7−6.0 min (65% A, 35% B). The flow rate was 350 μL/min. The temperatures of the column oven and autosampler tray were set at 40 and 10 °C, respectively.

The UPLC column effluent was interfaced to a Xevo TQ-S MS/MS system, equipped with an electrospray ionization (ESI) probe operating in the negative mode (all from Waters). A divert valve was used, and the UPLC effluent was directed to the mass spectrometer from 1.5 to 3.5 min.

Instrument parameters were optimized by direct infusion of working solutions of 1.0 μg/mL of OTA and the IS at a flow-rate of 10 μL/min in combination with the mobile phase (50% A, 50% B, flow-rate: 200 μL/min). The following parameters were used: capillary voltage, 3.0 kV; cone, 30 V; source offset, 50 V; desolvation temperature, 550 °C; desolvation gas, 900 L/h; cone gas, 150 L/h; nebulizer pressure, 7.0 bar; LM resolution 1 and 2, 2.72 and 2.86, respectively; HM resolution 1 and 2, 14.90 and 15.15, respectively; ion energy 1 and 2, 0.2 and 0.7, respectively; collision gas flow, 0.15 mL/min. MS/MS acquisition was performed in the SRM mode, and the following transitions (m/z) were used as quantifier and qualifier ion, respectively, for OTA, 402.0 > 358.0 and 402.0 > 167.0, and for the IS, 422.0 > 377.1 and 422.0 > 175.0.

Corresponding matrix-matched calibration curves were used for quantification of OTA in each aliquot. Plasma protein binding was calculated at each level (0.5, 1, and 5 μg/mL) using the following equation:

$$\text{Plasma protein binding (\%)} = (C_{\text{aliquot1}} - C_{\text{av aliquot2and3}})/(C_{\text{aliquot1}}) \times 100$$

DOI: 10.1021/acs.jafc.7b06048
J. Agric. Food Chem. 2018, 66, 2129−2135

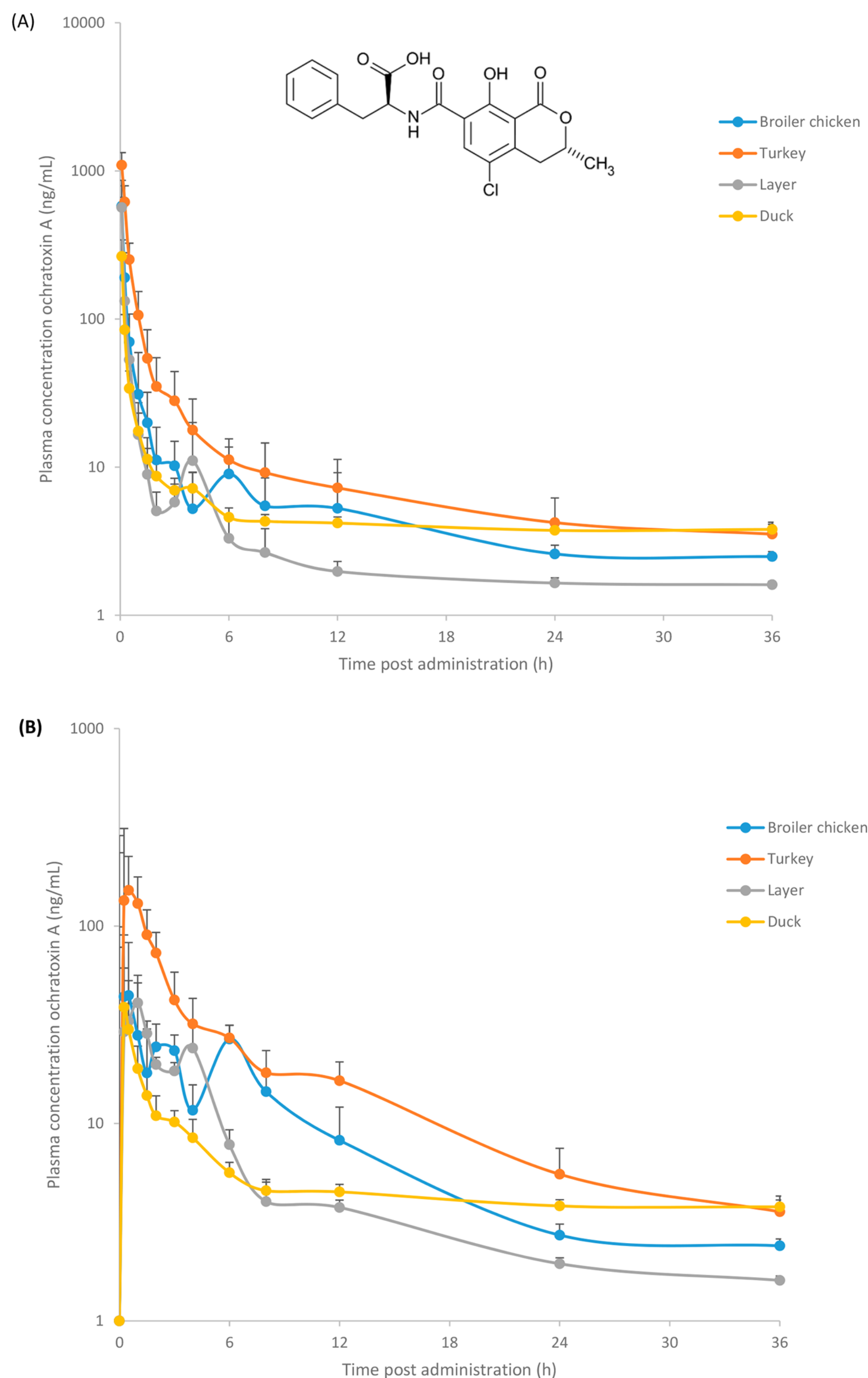

Figure 1. Plasma concentration−time profile of ochratoxin A (OTA) after single (A) intravenous and (B) oral administration of 0.25 mg OTA/kg body weight to broiler chickens, turkeys, layers, and Muscovy ducks (n = 4♂/4♀). Values are presented as mean + standard deviation. The insert in panel A is the chemical structure of OTA.

Toxicokinetic Analysis. Noncompartmental toxicokinetic analysis of OTA was performed with Phoenix (Certara, Princeton, NJ, USA). The following toxicokinetic parameters were calculated for IV and PO administration: maximal plasma concentration for PO (C_{max}), plasma concentration at time 0 for IV (C_0), time to maximal plasma concentration (T_{max}), area under the plasma concentration−time curve from time 0 to 36 h (AUC_{0-t}), elimination rate constant (k_{el}), elimination half-life ($T_{1/2el}$), total body clearance (Cl), and volume of distribution (V_d). The AUC was determined using the trapezoidal method (linear up-log down). The Cl and V_d after PO administration were not corrected for oral bioavailability and were expressed as V_d/F and Cl/F.

The absolute oral bioavailability (expressed as %, F%) for each individual bird was calculated according to the formula:

$$F\% = AUC_{0-36h\,PO}/AUC_{0-36h\,IV} \times 100$$

Statistical Analysis. All toxicokinetic parameters for each administration route were compared between animal species and

DOI: 10.1021/acs.jafc.7b06048
J. Agric. Food Chem. 2018, 66, 2129−2135

Table 1. Main Toxicokinetic Parameters of Ochratoxin A after Single Oral (PO) and Intravenous (IV) Administration (0.25 mg/kg BW) to Broiler Chickens, Layers, Turkey Poults, and Muscovy Ducks (n = 4♂ and 4♀)[a]

	broiler chickens				layers				turkeys				ducks			
	IV		PO		IV		PO		IV		PO		IV		PO	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
C_{max} (PO) or C_0 (IV) (ng/mL)	910.52 ± 627.31[a]	1203.86 ± 379.68[a]	47.48 ± 18.07[a]	75.06 ± 48.04[a,b]	1440.46 ± 584.34[a]	1104.76 ± 309.63[a]	55.52 ± 15.65[a]	49.16 ± 15.65[a]	1430.45 ± 276.19[a]	1477.02 ± 312.60[a]	201.02 ± 97.06[b]	178.99 ± 44.65[b]	657.08 ± 139.49[b]	307.52 ± 119.41[b]	47.98 ± 18.92[a]	34.87 ± 4.58[a]
T_{max} (h)			4.63 ± 2.06[a]	1.43 ± 1.06[a,b]			0.75 ± 1.13[b,c]	1.88 ± 1.13[b,c]			0.81 ± 0.59[b,c]	0.75 ± 0.25[b]			0.31 ± 0.09[c]	0.31 ± 0.09[c]
$AUC_{0-36\ h}$ (h ng/mL)	335.57 ± 79.30[a]	277.20 ± 15.32[a]	301.43 ± 42.71[a]	303.64 ± 37.67[a]	233.91 ± 9.23[b]	221.56 ± 14.15[b]	198.71 ± 21.73[b]	217.19 ± 21.73[b]	584.08 ± 84.97[c]	692.98 ± 73.40[c]	610.84 ± 88.60[c]	679.12 ± 72.66[c]	200.92 ± 17.29[b]	185.02 ± 14.74[b]	208.41 ± 20.52[b]	173.36 ± 23.67[b]
Cl (IV) or Cl/F (PO) (L/h/kg)	0.61 ± 0.12[a]	0.74 ± 0.10[a]	0.71 ± 0.17[a]	0.77 ± 0.17[a]	0.82 ± 0.21[a]	0.98 ± 0.28[a]	1.03 ± 0.10[a]	0.89 ± 0.10[a]	0.38 ± 0.04[b]	0.29 ± 0.02[b]	0.39 ± 0.06[b]	0.33 ± 0.02[b]	0.74 ± 0.25[a]	0.71 ± 0.27[a]	0.61 ± 0.18[a]	0.43 ± 0.25[a]
k_{el} (h^{-1})	0.045 ± 0.021[a]	0.068 ± 0.038[a]	0.061 ± 0.019[a,b]	0.096 ± 0.033[a]	0.084 ± 0.036[a]	0.058 ± 0.015[a]	0.035 ± 0.010[b,c]	0.043 ± 0.010[a,b]	0.044 ± 0.016[a]	0.078 ± 0.032[a]	0.074 ± 0.012[a]	0.057 ± 0.019[a,b]	0.041 ± 0.013[a]	0.062 ± 0.042[a]	0.020 ± 0.008[c]	0.017 ± 0.004[c]
$T_{1/2el}$ (h)	23.95 ± 15.27[a]	22.20 ± 19.72[a]	14.11 ± 6.45[a,b]	8.20 ± 1.98[a]	12.15 ± 7.60[a]	14.21 ± 5.89[a]	21.15 ± 3.91[b]	17.30 ± 3.91[b]	18.19 ± 6.18[a]	11.28 ± 4.92[a]	9.85 ± 1.71[a]	15.50 ± 7.28[a,b]	17.04 ± 1.44[a]	16.76 ± 7.47[a]	35.14 ± 19.46[b]	39.02 ± 18.45[b]
V_d (IV) or V_d/F (PO) (L/kg)	19.58 ± 11.74[a,c]	19.84 ± 14.33[a,c]	14.42 ± 5.88[a]	9.30 ± 2.77[a]	16.16 ± 9.40[a]	19.41 ± 6.38[a]	30.82 ± 16.52[a]	30.00 ± 16.51[a]	10.31 ± 4.34[a,b]	4.84 ± 2.27[b]	5.51 ± 0.91[b]	7.40 ± 3.58[c,b]	28.16 ± 9.42[a]	29.26 ± 13.61[a]	35.11 ± 4.89[a]	38.38 ± 5.88[a]
F (%)	100	100	93.31 ± 14.85[a]	109.60 ± 13.54[a]	100	100	84.69 ± 4.88[a]	98.91 ± 11.72[a]	100	100	109.95 ± 29.75[a]	88.01 ± 19.79[a]	100	100	103.87 ± 8.39[a]	93.88 ± 13.85[a]

[a]Values are presented as mean ± SD. C_{max}, maximal plasma concentration; C_0, plasma concentration at time 0; T_{max}, time to maximal plasma concentration,; AUC_{0-t}, area under the plasma concentration−time curve from time 0−36 h; Cl, total body clearance; Cl/F, total body clearance uncorrected for oral bioavailability; k_{el}, terminal elimination rate constant; $T_{1/2el}$, elimination half-life; V_d, volume of distribution; V_d/F, volume of distribution uncorrected for oral bioavailability; F, absolute oral bioavailability. A different superscript denotes a significant difference between animal species and gender for each administration route at $p < 0.05$.

DOI: 10.1021/acs.jafc.7b06048
J. Agric. Food Chem. 2018, 66, 2129−2135

gender using one-way analysis of variance (ANOVA). Plasma protein binding for each concentration level was compared between animal species and gender using one-way ANOVA as well. Homogeneity of variances was first evaluated using the Levene's test. When variances were not homogeneous, data were log-transformed (SPSS 24.0, IBM, USA). The level of significance was set at 0.05.

RESULTS AND DISCUSSION

No clinical symptoms were observed after PO or IV administration of OTA to the birds. In Figure 1, the plasma concentration−time profiles of OTA after IV and PO administration to broiler chickens, layers, turkeys, and ducks are depicted. The plasma concentration−time profiles reveal a secondary peak after oral as well as intravenous administration (4−6 h), which is most pronounced in broiler chickens and layers. This could be due to enterohepatic recycling of OTA, which has previously been demonstrated in calves, rats, and mice.[22−25] This was not observed in pigs and rabbits by Galtier et al.[19] This again demonstrates the species dependent toxicokinetics of OTA. Deconvolution techniques as well as curve fitting using a modified customary compartmental model were attempted, but no satisfactory results could be obtained for the PO data sets in both chicken species. Most available literature reports on OTA toxicokinetics are generated using a one- or two-compartmental model with first order absorption and elimination. However, this neglects the fact that OTA is actively absorbed from the gastro-intestinal tract and excreted in the kidney via transporters. These transporters in different peripheral organs could also contribute to toxicokinetic differences between animal species and also gender specific disposition. Therefore, three- or even four-compartmental models should be more appropriate.[26] To overcome these issues, a noncompartmental approach was used for all animals in which the data are used as such (no fitting or smoothing).

The values of the major toxicokinetic parameters are presented in Table 1. There were no significant differences observed between male and female birds for any of the parameters. In contrast, rats seem to have a gender specific absorption and disposition of OTA.[27,28] More specifically, males display a lower C_{max} and oral bioavailability than female rats. The presented results reveal that OTA has a complete oral bioavailability in the selected bird species (88.0−109.6%). It must be mentioned that the absorption of OTA can decrease with feed intake.[28] In the present study, birds were fasted prior to oral gavage, and the oral F could be lower in the field situation, around 40% in fed chickens,[19] where animals are not feed deprived.

The values of V_d are high (4.84−38.38 L/kg BW) and in contrast with the high plasma protein binding of OTA. These unexpectedly high V_d values are related to the computation method of V_d (or V_d/F) in noncompartmental modeling, namely $V_d = D/(k_{el}\,\text{AUC})$ with the terminal elimination rate constant being very low due to plasma persistence of OTA. When calculating the V_d after IV administration using $V_d = D/C_0$, more realistic values are obtained. Namely for broiler chickens, 0.27 ± 0.12 L/kg; layers, 0.23 ± 0.08 L/kg; turkeys, 0.18 ± 0.04 L/kg; and ducks, 0.76 ± 0.44 L/kg. No significant gender differences were found. Ducks have a significantly higher V_d (and consequently lower C_0) compared to the other bird species. Ducks might therefore be more susceptible to the effects of OTA given the higher tissue distribution. On the other hand, turkeys have a higher systemic exposure of OTA (based on AUC_{0-t}) due to their lower V_d and lower Cl (Table 1). Interestingly, the relatively higher V_d in ducks and lower V_d in turkeys are well related to the species-dependent differences in plasma protein binding of OTA, namely ranging between 82.2 and 88.9% in ducks and between 96.5 and 98.8% in turkeys (Table 2). No significant gender differences were found for plasma protein binding in any of the avian species. Being a hybrid parameter of V_d and Cl, also the elimination half-life depends inter alia on the affinity of OTA for and capacity of plasma proteins such as albumin. As mentioned above, albumin binds OTA with unusually high affinity. In humans, 99.8% of OTA is bound to albumin.[29] The primary OTA binding site on serum albumin in humans is the Sudlow's Site I (located on subdomain IIA ().[5] Binding to albumin can significantly impact the OTA toxicokinetics since in rats, who were albumin deficient, the total body clearance was 20−70-fold higher than in nondeficient rats.[30] Albumin binding strongly limits glomerular filtration of OTA; however, the small filtrated fraction can be reabsorbed in the tubule cells. Organic anion transporters (OATs) belong to the solute carrier superfamily (SLC) and the apical OAT4 transporter plays a role in the urinary OTA reabsorption, which results in persistence, renal accumulation, and toxicity.[9] There is a wide range in elimination half-life of OTA between species, for example, 24−39 h in mice, 55−120 h in rats, 72−120 h in pigs, 510 h in one macaque, and 840 h in a human volunteer, whereas in broiler chickens, the elimination half-life is rather short (4.1 h).[19,31] These differences can again be related to the affinity of OTA to serum albumin. More specifically, porcine serum albumin binds OTA with a higher affinity constant (71 100 M^{-1}) than chicken (50 700 M^{-1}) serum albumin.[32] The terminal elimination half-life in the presented study ranges between 8.20 and 39.02 h. The $T_{1/2el}$ of broiler chickens (22.20−23.95 h) is considerably longer than described by Galtier et al.[19] and can in part be explained by the sampling strategy and the quantification limits of the analytical methodology used. The last sampling point in the study of Galtier et al.[19] was at 4 h postadministration (LOQ = 200 ng/mL), whereas our last sampling point was at 36 h post administration (LOQ = 5.0 ng/mL), and the $T_{1/2el}$ was determined on the terminal phase of the elimination curve from 4 h p.a. onward. Ducks have a longer terminal $T_{1/2el}$, which begins 6−8 h post administration (see Table 1 and Figure 1) compared to the other birds species. This can be attributed to their higher V_d (Table 1)

Table 2. Plasma Protein Binding of Ochratoxin A (OTA) at Three Concentration Levels (0.5, 1, and 5 μg OTA/mL Plasma) in Broiler Chickens, Layers, Turkey Poults, and Muscovy Ducks (n = 3 ♂ and 3 ♀)[a]

concentration level (μg/mL)	broiler chickens (%)		layers (%)		turkeys (%)		ducks (%)	
	♂	♀	♂	♀	♂	♀	♂	♀
0.5	93.42 ± 5.44[a,b]	97.01 ± 1.93[a]	88.26 ± 1.15[a,b]	89.25 ± 0.59[a,b]	97.01 ± 0.28[a]	96.53 ± 0.15[a]	83.51 ± 2.15[b]	82.19 ± 2.84[b]
1	90.11 ± 7.47[a,b]	93.33 ± 2.01[a,b]	90.00 ± 1.47[a,b]	91.74 ± 0.79[a,b]	97.98 ± 0.09[a]	97.45 ± 0.07[a,b]	84.75 ± 2.00[a,b]	82.80 ± 2.81[b]
5	91.52 ± 1.83[a,b]	91.39 ± 1.84[a,b]	92.79 ± 1.41[a,b]	94.02 ± 0.76[a,b]	98.77 ± 0.08[a]	98.39 ± 0.23[a]	88.93 ± 2.13[b]	86.27 ± 2.22[b]

[a]Values are presented as mean ± SD. A different superscript denotes a significant difference between animal species and gender for each concentration level at $p < 0.05$.

DOI: 10.1021/acs.jafc.7b06048
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as $T_{1/2el}$ is a hybrid parameter of V_d and Cl. Clearance of OTA is lower in turkeys, which probably explains their higher LD_{50} value (and therefore lower toxicity) as previous studies demonstrated that OTA is metabolized into derivatives that are genotoxic and damage DNA.[33]

In conclusion, this is the first paper describing the absolute oral bioavailability, disposition, and plasma protein binding of OTA in different avian species, namely broiler chickens, layers, turkeys, and Muscovy ducks. Turkeys have a significantly lower Cl compared to other avian species and might be attributed to differences in biotransformation. The V_d was quite low (<1 L/kg) with ducks having the relatively highest V_d, and consequently longest $T_{1/2el}$, and turkeys the lowest V_d. The differences in V_d and elimination half-life can be related to, although high in all species, significant species-dependent differences in plasma protein binding. Future research can focus on a more in depth analysis of species related differences in biotransformation and expression of OATs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b06048.

Feed analyzed for mycotoxin contamination (PDF)

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Funding

G.A. is supported by a postdoctoral fellowship from the Research Foundation − Flanders.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The technical assistance of Julie Muyle and Maarten Claes is greatly appreciated. Software license for Phoenix was provided by Certara as part of their Centers of Excellence program.

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DOI: 10.1021/acs.jafc.7b06048
J. Agric. Food Chem. 2018, 66, 2129−2135