Supplementary Materials: Assessment of Dried Blood Spots for Multi-Mycotoxin Biomarker Analysis in Pigs and Broiler Chickens

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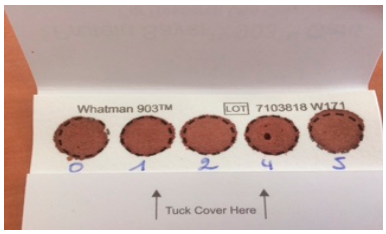


Figure S1. Example of a Whatman® 903 protein saver card spotted with 60 µL of blood.

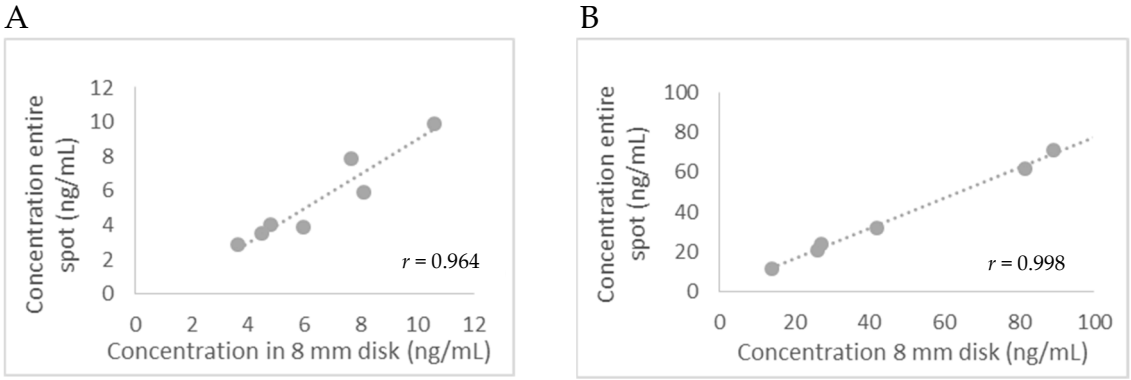


Figure S2. Comparison of mycotoxin concentrations upon extraction of the entire blood spot and the 8 mm disk in DBS obtained after administration of (**A**) DON (36 µg·kg^{-1} BW) and (**B**) AFB1 (0.1 mg·kg^{-1} BW) to pigs (n = 2).

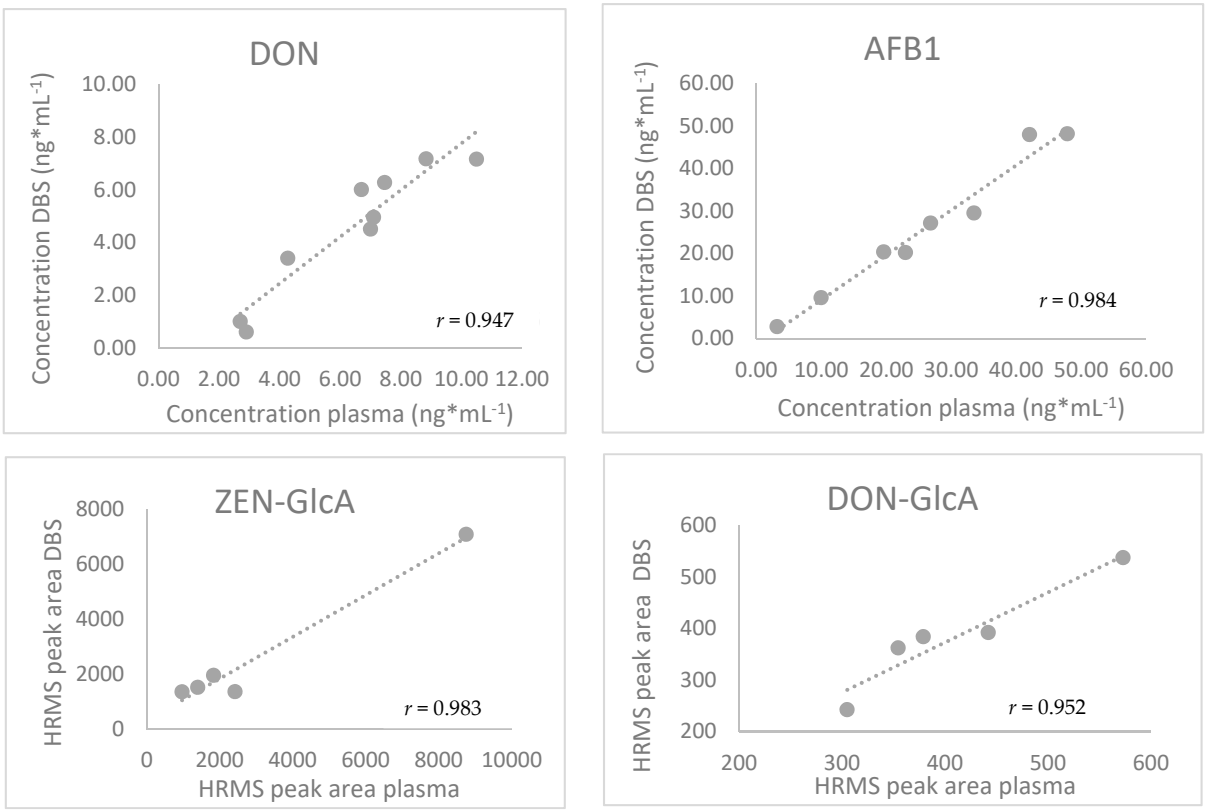


Figure S3. Comparison of mycotoxin concentrations determined after LC-MS/MS analysis of dried blood spots and plasma samples obtained after a single intra-gastric bolus administration of deoxynivalenol (DON) (36 µg·kg^{-1} BW), aflatoxin B1 (AFB1) (0.1 mg·kg^{-1} BW) and zearalenone (ZEN) (3 mg·kg^{-1} BW) to pigs (n = 2).

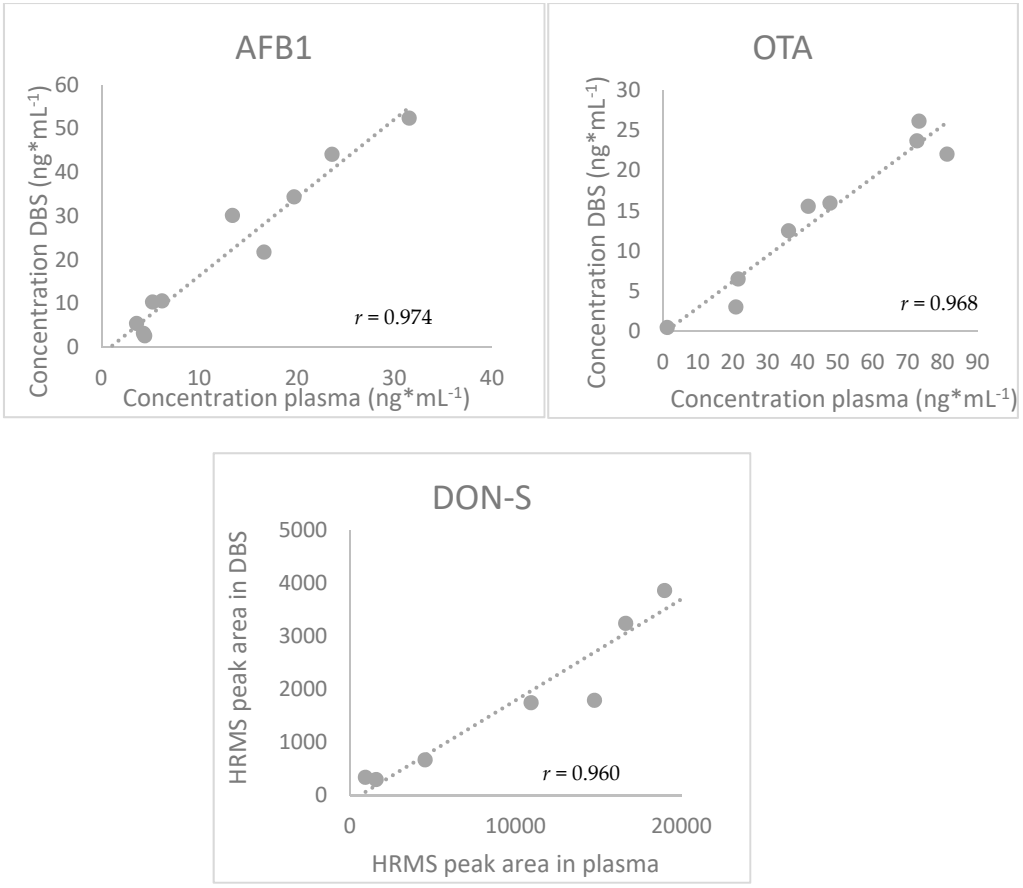


Figure S4. Comparison of mycotoxin concentrations determined after LC-MS/MS analysis of dried blood spots and plasma samples obtained after single intra-crop bolus administration of ochratoxin A (OTA) (0.25 mg·kg^{-1} BW), aflatoxin B1 (AFB1)(2 mg·kg^{-1} BW) and deoxynivalenol (DON)(0.5 mg·kg^{-1} BW) to broiler chickens (n = 2).

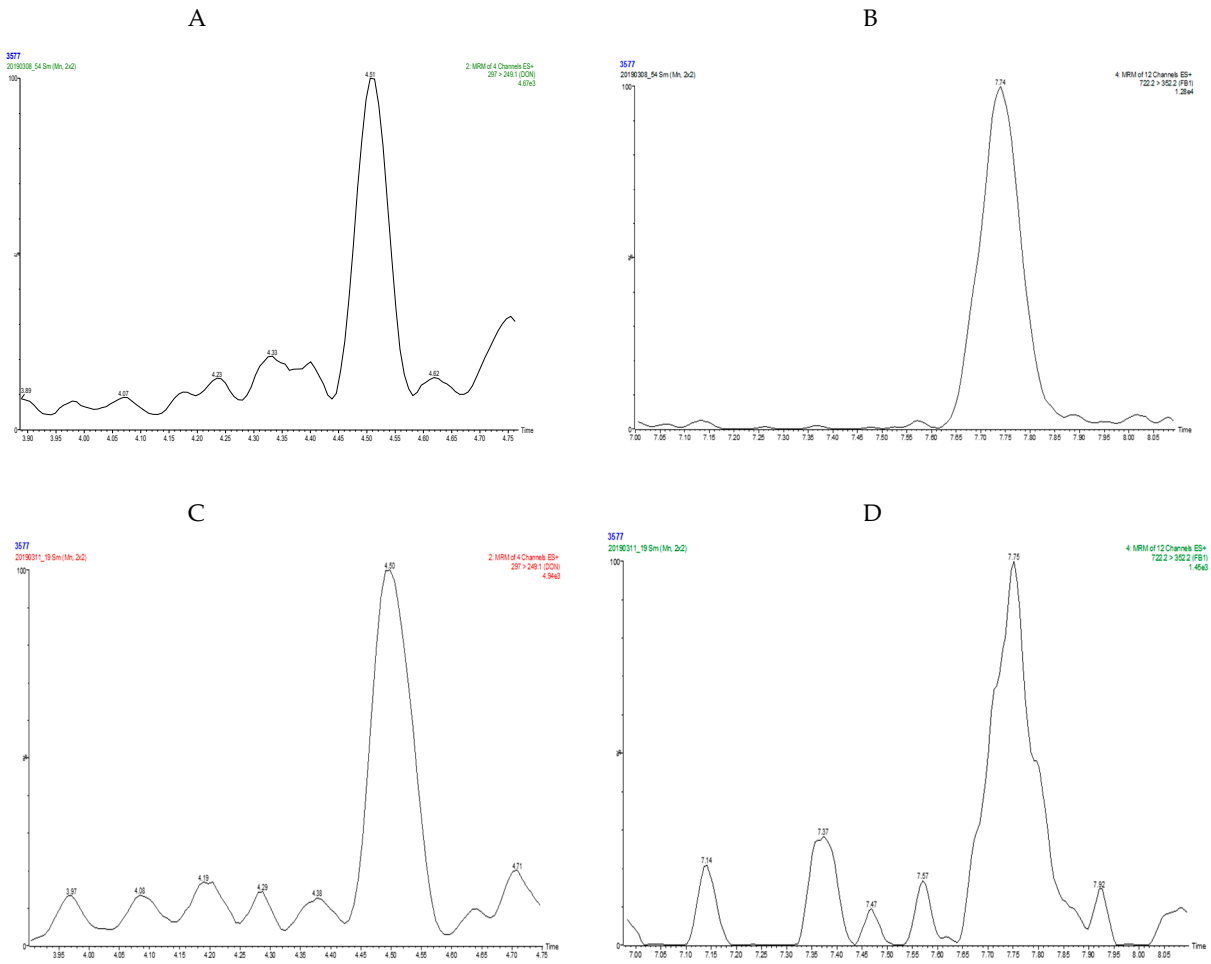


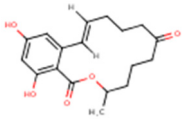
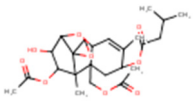
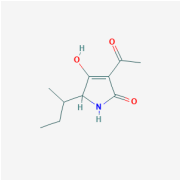
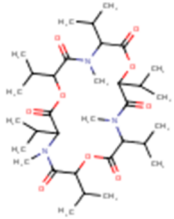
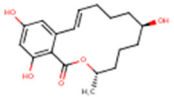
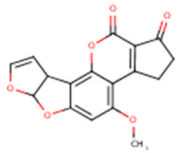
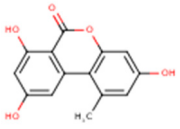
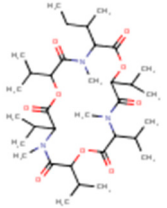
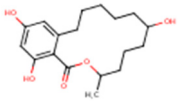
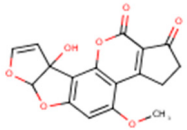
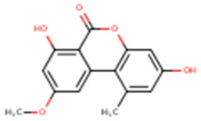
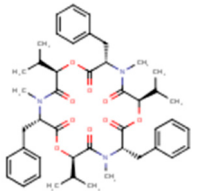
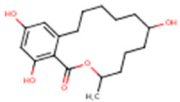
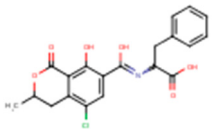
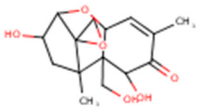
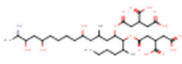
Figure S5. Chromatograms of FB1 and DON in plasma and DBS of sows suffering from postpartum problems obtained in an exposure assessment study performed in 5 pig farms. (**A** and **B**) show the presence of DON (2.07 ng/mL) and FB1 (50 ng/mL), respectively in dried blood spots and (**C** and **D)** the presence of DON (<LOQ) and FB1 (not quantified) in plasma, respectively.

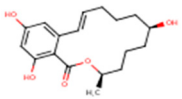
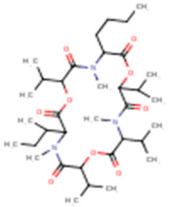
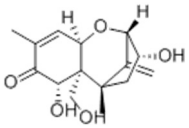
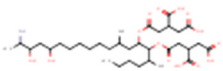
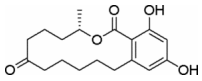
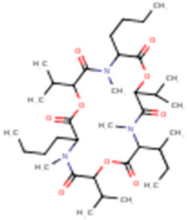
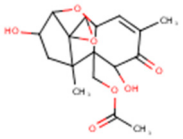
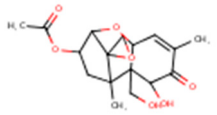
Table S1. Overview of the parent mycotoxins and possible phase I and II metabolites included in the LC-MS/MS and LC-HRMS analysis described by Lauwers et al [3].

Name	Name
Deoxynivalenol	$^{13}C_{15}$-Deoxynivalenol
De-epoxy-deoxynivalenol	$^{13}C_{17}$-Aflatoxin B1
3/15-acetyldeoxynivalenol	$^{13}C_{20}$-Ochratoxin A
T2-toxin	$^{13}C_{24}$-T2-toxin
HT2-toxin	$^{13}C_{34}$-Fumonisin B1
Aflatoxin B1	$^{15}N_3$-Enniatin B
Aflatoxicol	$^{13}C_6{}^{15}$N-Tenuazonic acid
Aflatoxin M1	$^{13}C_{18}$-Zearalenone
Ochratoxin A	Deoxynivalenol-glucuronide
Enniatin A1	De-epoxy-deoxynivalenol glucuronide
Enniatin A	Deoxynivalenol-sulphate
Enniatin B	Deoxynivalenol-di-sulphate
Enniatin B1	3/15-acetyl-deoxynivalenol-sulphate
Beauvericin	Ochratoxin alfa
Fumonisin B1	Zearalenone-glucuronide

Fumonisin B2	Zearalenone-di-glucuronide
Tenuazonic acid	α/β-zearalenol- or zearalanone-glucuronide
Alternariol	Zearalenone-sulphate
Alternariol methyl ether	α/β-zearalenol- or zearalanone-sulphate
Zearalenone	α/β-zearalenol-sulphate
Zearalanone	Tenuazonic acid-sulphate
α-Zearalenol	Alternariol-sulphate
α-Zearalanol	Alternariol-methyl ether sulphate
β-Zearalanol	
β-Zearalenol	

Table S2. Overview of the structure of the parent mycotoxins and phase I metabolites included in the analysis.

Analyte	Chemical Structure	Analyte	Chemical Structure	Analyte	Chemical Structure	Analyte	Chemical Structure
ZEN	 $C_{18}H_{22}O_5$	T2	 $C_{24}H_{34}O_9$	TEA	 $C_{10}H_{15}NO_3$	ENNB	 $C_{33}H_{57}N_3O_9$
AZEL	 $C_{18}H_{24}O_5$	AFB1	 $C_{17}H_{12}O_6$	AOH	 $C_{14}H_{10}O_5$	ENNB1	 $C_{34}H_{59}N_3O_9$
AZAL	 $C_{18}H_{26}O_5$	AFM1	 $C_{17}H_{12}O_7$	AME	 $C_{15}H_{12}O_5$	BEA	 $C_{45}H_{57}N_3O_9$
BZAL	 $C_{18}H_{26}O_5$	OTA	 $C_{20}H_{18}ClNO_6$	DON	 $C_{15}H_{20}O_6$	FB1	 $C_{34}H_{59}NO_{15}$

BZEL		ENN A1		DOM-1		FB2	
$C_{18}H_{24}O_5$		$C_{35}H_{61}N_3O_9$		$C_{15}H_{20}O_5$		$C_{34}H_{59}NO_{14}$	
ZAN		ENNA		15-ADON		3-ADON	
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