

Supplementary

# Strong Alterations in the Sphingolipid Profile of Chickens Fed a Dose of Fumonisin Considered Safe

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**Table S1.** Linearity of the method in net solvent and recovery of 33 standards measured in liver.

Analyte <sup>1</sup>	Net solvent			Liver														
	Conc <sup>2</sup>	a	(R <sup>2</sup> )	Conc <sup>3</sup>	R <sup>4</sup>	RSD <sup>5</sup>	Conc <sup>3</sup>	R <sup>4</sup>	RSD <sup>5</sup>	Conc <sup>3</sup>	R <sup>4</sup>	RSD <sup>5</sup>	Conc <sup>3</sup>	R <sup>4</sup>	RSD <sup>5</sup>	Conc <sup>3</sup>	R <sup>4</sup>	RSD <sup>5</sup>
1dSo	0.1-12	1.0833	0.9998	16	81	9	31	75	8	63	73	8	125	71	8	250	83	15
1dSa	0.1-12	1.074	0.9999	16	99	14	31	100	33	63	105	17	125	85	12	250	91	9
d18:1 (So)	125-4000	1.0015	0.9985	5000	115	34	10000	111	8	20000	111	6	40000	120	8	80000	91	12
d18:0 (Sa)	16-1000	1.077	0.9949	625	157	15	1250	169	21	2500	172	5	5000	150	4	10000	135	4
18:1/2:0	2-125	0.9911	0.9998	78	104	26	156	105	17	313	104	14	625	103	5	1250	103	4
18:0/2:0	2-250	0.9898	0.9996	156	122	6	313	106	10	625	123	18	1250	115	8	2500	115	10
d18:1_P	63-1000	0.9982	0.9994	1250	141	57	2500	103	40	5000	100	9	10000	110	13	20000	139	6
d18:0_P	63-1000	1.0155	0.9925	1250	123	16	2500	124	11	5000	119	21	10000	146	7	20000	171	6
GluSo	1-125	1.0064	0.9998	156	110	22	313	120	10	625	107	13	1250	108	13	2500	126	3
LysoSM	1-63	1.0018	0.9998	78	107	21	156	112	12	313	115	7	625	108	7	1250	113	6
18:1/14:0	4-250	0.9186	0.9975	313	119	8	625	100	8	1250	105	8	2500	107	8	5000	81	14
18:1/16:0	500-32000	1.0013	1	80000	82	19	160000	88	16	320000	89	20	640000	85	13			
18:0/16:0	125-4000	0.8859	0.9987	10000	112	11	20000	110	9	40000	129	13	80000	123	21			

18:1/18:0	125-4000	0.9913	0.9993	10000	97	8	20000	98	25	40000	108	14	80000	72	14			
18:1/20:0	125-2000	1	0.9997	5000	115	25	10000	131	19	20000	128	19	40000	119	22			
18:1/16:0_P	125-2000	1.0004	0.9996	5000	24	16	10000	40	11	20000	54	2	40000	81	17	80000	78	12
18:1/22:0	1000-16000	1.0018	0.9994	40000	82	20	80000	91	9	160000	75	12	320000	76	17			
LacSo	1-63	1.0003	0.9999	39	85	12	78	111	16	156	108	14	313	114	9	625	100	8
18:1/24:1	1000-16000	0.9834	0.9978	40000	89	23	80000	94	17	160000	67	18	320000	65	20			
18:1/24:0	125-8000	0.9897	0.9985	20000	64	14	40000	70	9	80000	67	8	160000	66	20			
18:0/24:0	8-2000	0.9918	0.9992	2500	35	22	5000	47	21	10000	53	15	20000	55	14	40000	74	6
SM18:1/14:0	2-500	0.9098	0.9974	156	71	27	625	79	10	1250	70	7	2500	72	3	5000	91	9
Glu18:1/16:0	63-4000	0.9846	0.9983	2500	81	10	5000	92	18	10000	75	14	20000	82	5	40000	87	6
SM18:1/16:0	500-16000	0.9753	0.999	20000	94	33	40000	60	16	80000	60	10	160000	86	5	320000	65	15
SM18:1/18:1	4-500	1.0131	0.9995	313	56	20	625	55	30	1250	57	6	2500	52	10	5000	51	2
SM18:1/18:0	500-32000	0.975	0.9987	20000	120	14	40000	103	6	80000	88	3	160000	100	8	320000	105	11
SM18:1/20:0	125-4000	1	0.9998	20000	112	34	40000	80	11	80000	78	3	160000	104	14	320000	78	11
SM18:1/22:0	250-16000	1.003	1	40000	69	18	80000	72	19	160000	106	19	320000	80	15			
Glu18:1/24:1	31-1000	1.0035	0.9988	2500	115	15	5000	84	29	10000	93	17	20000	98	10	40000	86	14
SM18:1/24:1	500-32000	1.009	0.9999	40000	109	32	80000	73	5	160000	80	6	320000	104	15	640000	104	12
SM18:1/24:0	63-4000	0.9926	0.9996	10000	78	27	20000	71	19	40000	98	14	80000	89	16			
Lac18:1/16:0	31-2000	1.001	0.9997	2500	118	24	5000	123	7	10000	87	15	20000	100	5	40000	96	11
Lac18:1/24:1	16-1000	0.9915	0.9993	1250	107	24	2500	139	5	5000	102	17	10000	108	8	20000	120	14

<sup>1</sup>Recovery (R) was calculated by correcting the concentrations measured by the R measured for each representative IS. The IS representative of the different standards were d17:1 for d18:1\_dOH and d18:1, d17:1 for d18:0\_dOH and d18:0; d17:1\_P for d18:1\_P; d17:0\_P for d18:0\_P; 18:1/12:0 for 18:1/14:0, 18:1/16:0, 18:0/16:0, and 18:1/18:0; 18:1/25:0 for 18:1/20:0, 18:1/22:0, 18:1/24:1, 18:1/24:0 and 18:0/24:0; SM18:1/12:0 for SM18:1/14:0, SM18:1/16:0, SM18:1/18:1, SM18:1/18:0, SM18:1/20:0, SM18:1/22:0, SM18:1/24:1, and SM18:1/24:0; Glu18:1/12:0 for Glu18:1/16:0 and Glu18:1/24:1; Lac18:1/12:0 for Lac18:1/16:0 and Lac18:1/24:1. No correction by IS was used for 18:1/2:0, 18:0/2:0, GluSo, LysoSM, and LacSo. <sup>2</sup>Conc = concentration expressed in pmol/mL, the ratio of each concentration tested was 2, n = 4 per concentration <sup>3</sup>Conc = concentration expressed in pmol/g, n = 4 <sup>4</sup>R = Recovery, expressed in %, n = 4. <sup>5</sup>RSD = relative standard deviation, expressed in %, n = 4.

**Table S2.** Linearity of the method for net solvent and recovery of 33 standards measured in plasma.

Analyte <sup>1</sup>	Net solvent			Plasma														
	Conc <sup>2</sup>	a	(R <sup>2</sup> )	Conc <sup>3</sup>	R <sup>4</sup>	RSD <sup>5</sup>	Conc <sup>3</sup>	R <sup>4</sup>	RSD <sup>5</sup>	Conc <sup>3</sup>	R <sup>4</sup>	RSD <sup>5</sup>	Conc <sup>3</sup>	R <sup>4</sup>	RSD <sup>5</sup>	Conc <sup>3</sup>	R <sup>4</sup>	RSD <sup>5</sup>
1dSo	0.1-12	1.0833	0.9998	2	88	12	4	86	5	8	76	6	16	73	1	31	82	5
1dSa	0.1-12	1.074	0.9999	2	67	20	4	64	16	8	63	10	16	68	5	31	78	9
d18:1 (So)	16-500	0.8317	0.9905	313	111	5	625	117	4	1250	119	4	2500	114	2			
d18:0 (Sa)	8-500	0.9953	0.9998	156	115	5	313	121	4	625	107	10	1250	98	2	2500	106	12
18:1/2:0	2-125	0.9911	0.9998	20	88	10	39	85	10	78	83	4	156	84	5	313	82	6
18:0/2:0	2-250	0.9898	0.9996	39	90	11	78	90	6	156	86	8	313	81	4	625	84	5
d18:1_P	63-1000	0.9982	0.9994	625	204	27	1250	227	8	2500	194	6	5000	172	7			
d18:0_P	63-1000	1.0155	0.9925	313	260	16	625	289	7	1250	278	3	2500	231	8	5000	258	7
GluSo	1-125	1.0064	0.9998	10	96	19	20	100	10	39	86	8	78	86	6	156	97	10
LysoSM	1-63	1.0018	0.9998	10	182	10	20	162	12	39	136	9	78	134	7	156	131	8
18:1/14:0	1-63	1.0138	0.9996	20	69	13	39	72	17	78	76	10	156	75	5	313	91	12
18:1/16:0	125-8000	0.9996	0.9999	2500	119	15	5000	112	12	10000	109	6	20000	98	13	40000	96	16
18:0/16:0	16-1000	0.9954	0.9993	313	91	17	625	84	5	1250	107	13	2500	97	9	5000	108	21
18:1/18:0	16-1000	0.9945	0.9999	313	108	11	625	91	9	1250	99	12	2500	89	8	5000	99	17
18:1/20:0	8-500	0.9891	0.9997	156	78	25	313	77	18	625	98	19	1250	88	7	2500	106	19
18:1/16:0_P	125-2000	1.0004	0.9996	313	74	37	625	98	15	1250	105	16	2500	111	4	5000	113	6
18:1/22:0	63-8000	0.9864	0.9986	1250	93	11	2500	81	15	5000	78	13	10000	73	8	20000	85	22
LacSo	1-63	1.0003	0.9999	10	145	8	20	122	18	39	103	18	78	91	7	156	91	17
18:1/24:1	125-8000	0.8936	0.9961	2500	99	27	5000	121	15	10000	87	14	20000	103	19	40000	130	9
18:1/24:0	63-4000	1.0093	0.9995	1250	115	20	2500	125	8	5000	100	5	10000	98	23	20000	111	4
18:0/24:0	8-2000	0.9918	0.9992	156	129	25	313	118	12	625	101	2	1250	84	14	2500	90	13
SM18:1/14:0	2-500	0.9098	0.9974	156	88	29	313	70	15	625	73	14	1250	87	4			
Glu18:1/16:0	31-1000	0.9986	0.9999	313	124	11	625	93	14	1250	74	10	2500	63	13	5000	77	20
SM18:1/16:0	500-16000	0.9753	0.999	10000	99	23	20000	73	30	40000	88	7						
SM18:1/18:1	4-500	1.0131	0.9995	156	62	43	313	69	15	625	55	5	1250	63	11	2500	68	5
SM18:1/18:0	500-32000	0.975	0.9987	5000	97	44	10000	84	18	20000	68	17	40000	70	15	80000	85	5
SM18:1/20:0	125-4000	1	0.9998	625	93	32	1250	90	8	2500	73	12	5000	76	20	10000	89	5
SM18:1/22:0	250-16000	1.003	1	2500	113	15	5000	93	12	10000	71	13	20000	73	21	40000	90	10
Glu18:1/24:1	31-1000	1.0035	0.9988	156	103	23	313	92	21	625	72	10	1250	52	13	2500	66	11
SM18:1/24:1	500-32000	1.009	0.9999	5000	100	17	10000	78	7	20000	69	14	40000	78	18	80000	85	9
SM18:1/24:0	63-4000	0.9926	0.9996	625	112	27	1250	93	6	2500	68	21	5000	69	28	10000	82	10

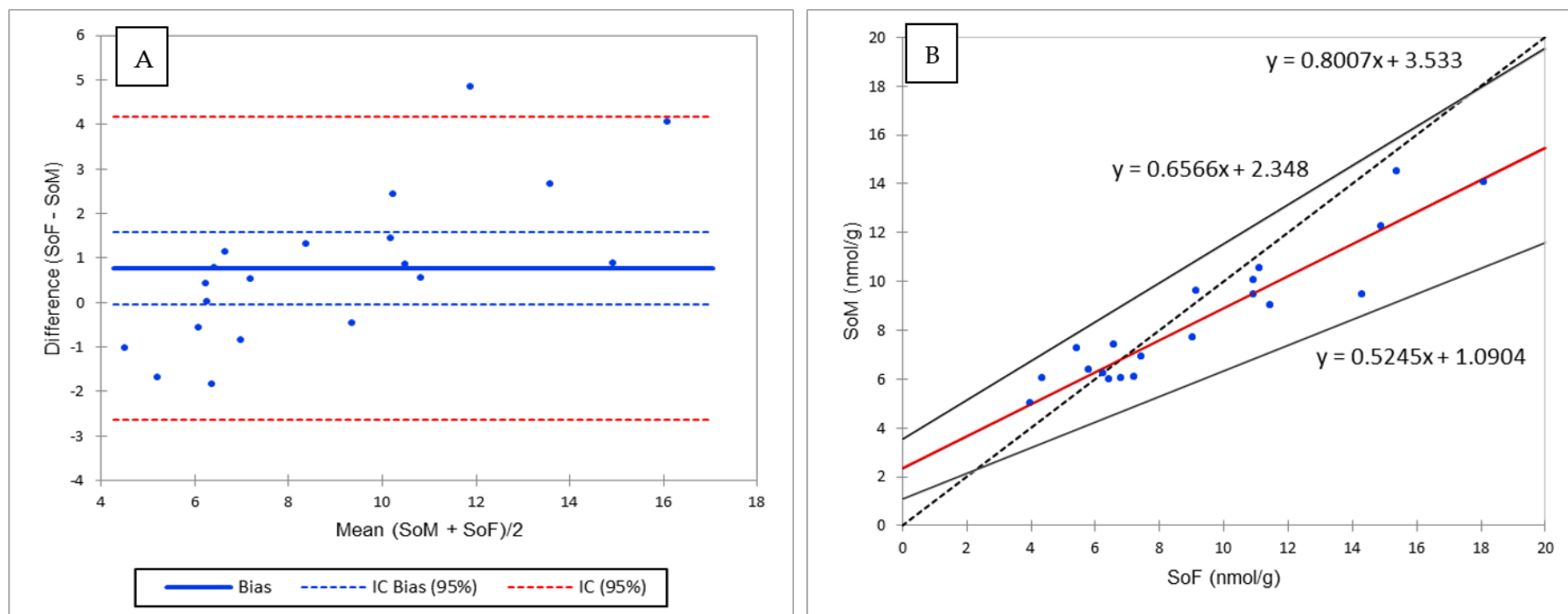
Lac18:1/16:0	31-2000	1.001	0.9997	313	97	9	625	79	22	1250	80	7	2500	65	8	5000	68	16
Lac18:1/24:1	16-1000	0.9915	0.9993	156	134	19	313	124	2	625	94	13	1250	72	16	2500	98	25

<sup>1</sup> Recovery (R) was calculated by correcting the concentrations measured with the R measured for each representative IS. The IS representative of the different standards were d17:1 for d18:1\_dOH and d18:1, d17:1 for d18:0\_dOH and d18:0; d17:1\_P for d18:1\_P; d17:0\_P for d18:0\_P; 18:1/12:0 for 18:1/14:0, 18:1/16:0, 18:0/16:0, and 18:1/18:0; 18:1/25:0 for 18:1/20:0, 18:1/22:0, 18:1/24:1, 18:1/24:0 and 18:0/24:0; SM18:1/12:0 for SM18:1/14:0, SM18:1/16:0, SM18:1/18:1, SM18:1/18:0, SM18:1/20:0, SM18:1/22:0, SM18:1/24:1, and SM18:1/24:0; Glu18:1/12:0 for Glu18:1/16:0 and Glu18:1/24:1; Lac18:1/12:0 for Lac18:1/16:0 and Lac18:1/24:1. No correction by IS was used for 18:1/2:0, 18:0/2:0, GluSo, LysoSM, and LacSo. <sup>2</sup> Conc = concentration expressed in pmol/mL, the ratio between each concentration tested is 2, n = 4 per concentration <sup>3</sup> Conc = concentration expressed in pmol/mL, n = 4. <sup>4</sup> R = Recovery, expressed in %, n = 4. <sup>5</sup> RSD = relative standard deviation, expressed in %, n = 4.

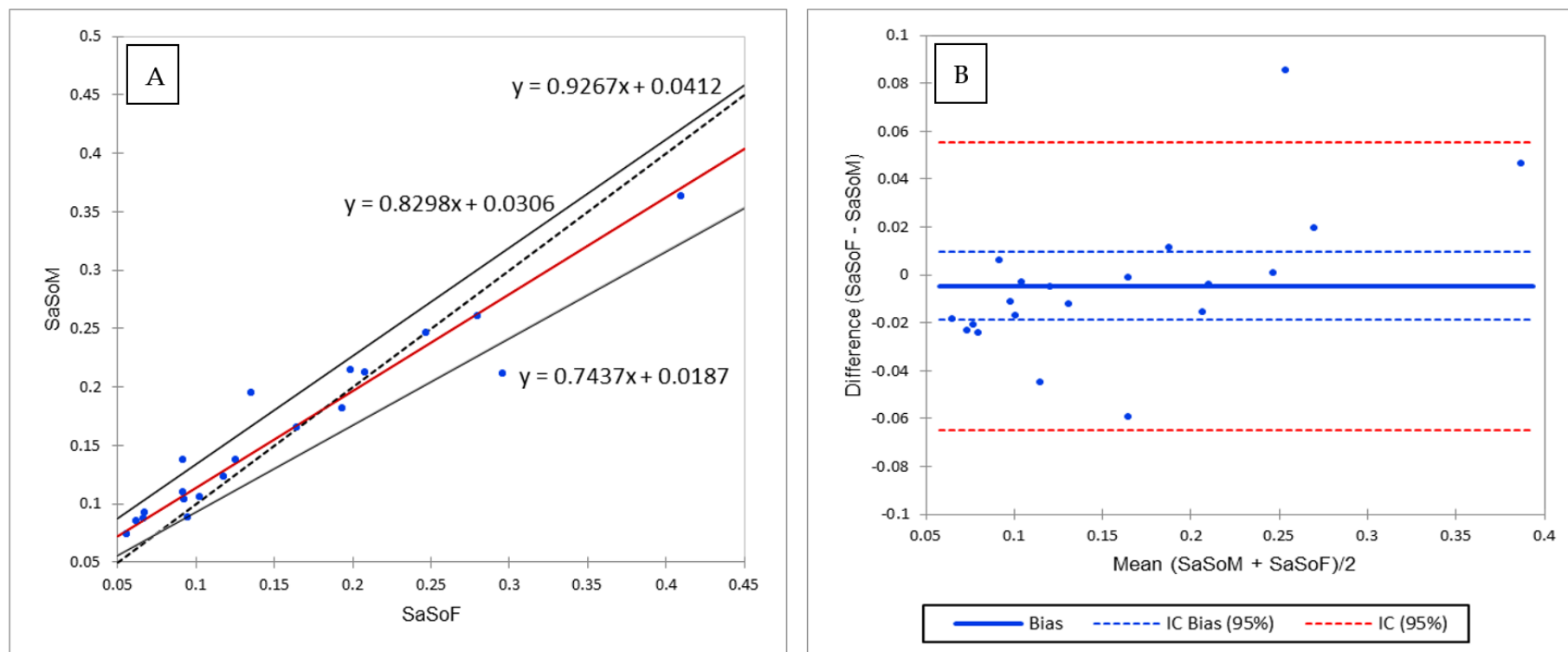
**Table S3.** Signal suppression and enhancement (SSE) of some analytes measured in liver and plasma<sup>1</sup>

Analyte	Liver			Plasma		
	Concentration <sup>2</sup>	SSE (%)	RSD (%)	Concentration <sup>2</sup>	SSE (%)	RSD (%)
d18:0 (Sa)	2500-20000	66	12	313-5000	67	16
d18:1_P	2500-20000	326	6	1250-5000	287	12
d18:0_P	2500-20000	470	10	625-5000	442	13
LysoSM	80-625	162	7	39-156	188	11
18:0/24:0	1250-20000	113	2	313-5000	103	3
SM18:1/18:1	625-10000	95	3	313-2500	97	11

<sup>1</sup>SSE was measured using the “one point area” method as described in materials and methods for the IS. <sup>2</sup>Range of concentration for the analytes spiked after the extraction, expressed in pmol/g liver or pmol/ml plasma. The concentration ratio between each level tested is 2, n=4 per concentration.



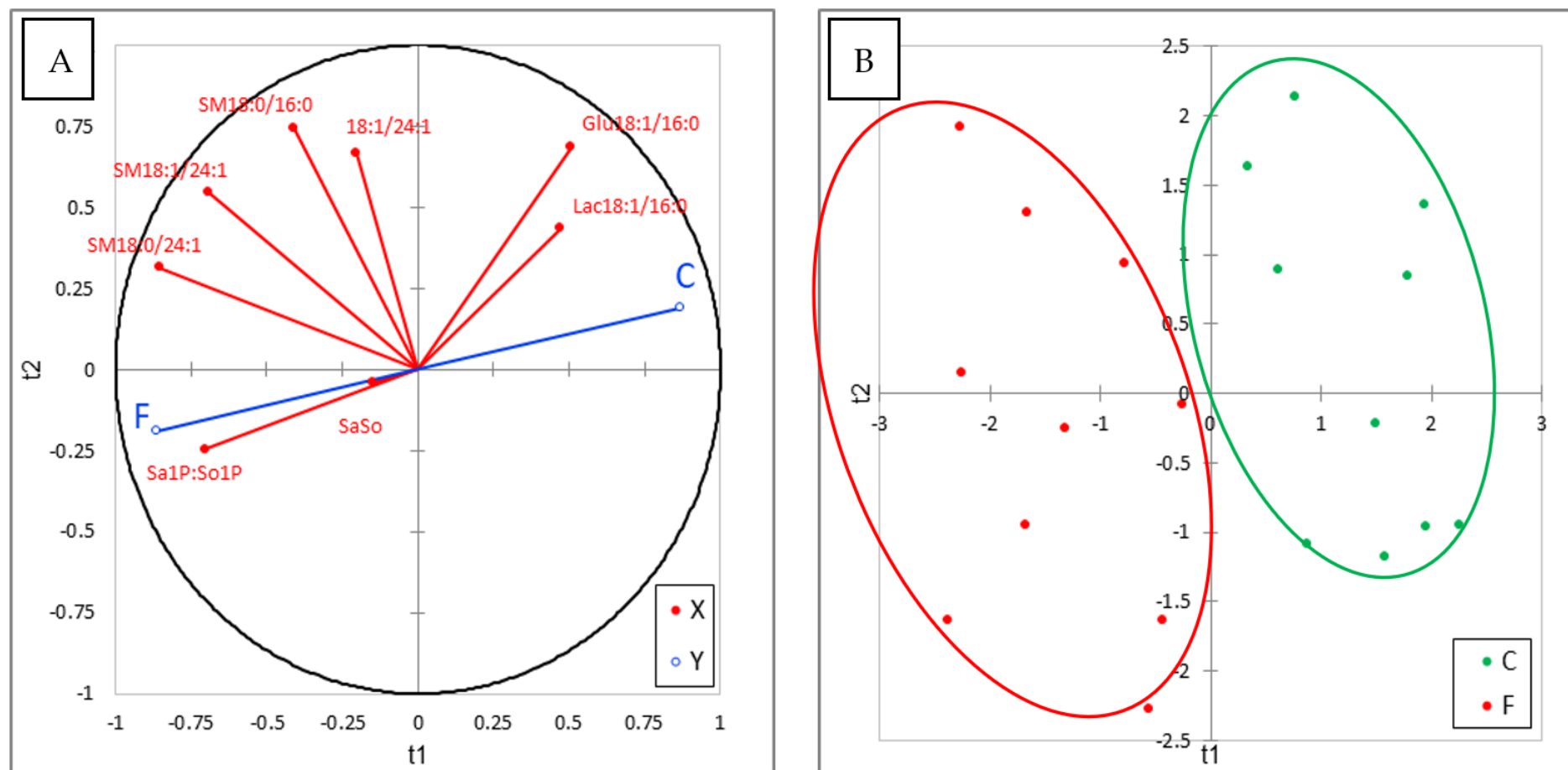
**Figure S1.** Comparison of sphingosine concentrations in liver measured by fluorescence detection (SoF) and mass detection (SoM). (A) Passing and Bablok regression; (B) Bland-Altman comparison.



**Figure S2.** Comparison of the sphinganine to sphingosine ratio in liver measured by fluorescence detection (SaSoF) and mass detection (SaSoM). (A) Passing and Bablok regression; (B) Bland-Altman comparison.

Confusion matrix for the training sample (Variable Groups) : Liver				
From \ To	V	Y	Total	% correct
V	10	0	10	100.00%
Y	0	10	10	100.00%
Total	10	10	20	100.00%
Confusion matrix for the training sample (Variable Groups) : plasma				
From \ To	V	Y	Total	% correct
V	10	0	10	100.00%
Y	0	10	10	100.00%
Total	10	10	20	100.00%

**Figure S3.** Confusion matrix for the training sample of the PLS regression performed on variables representative of the effect of FB on SL in liver and plasma.



**Figure S4.** Partial least square discriminant analysis (PLS-DA) performed on 6 SL, Sa:So ratio and Sa1P:So1P ratio measured in the livers of control chickens (C) not exposed to fumonisins and chickens fed for 9 days with 20 mg FB1+FB2/kg (F). (A) Correlation plot between the explanatory (X) and dependent (Y) variables. (B) Discrimination on the factor axes extracted from the original explanatory variables.  $R^2X = 0.567$ ,  $R^2Y = 0.787$ ,  $Q^2 = 0.735$ .