SUPPLEMENTARY MATERIAL

Table 1. Feed formulation (as % inclusion level) of two gilthead sea bream diets (GSB-D) with low or high inclusion levels of plant material (GSB-D1 and GSB-D2, respectively), and three production replicates for Atlantic salmon diets with similar high plant ingredient inclusion levels with feed ingredients from the same batch (AS-D1-3).

Ingredient (%)	Diets		
	GSB-D1	GSB-D2	AS-D1-3
Fish meal	23.00	3.00	8.00
Krill meal	-	-	2.00
SPC 60%	-	-	18.00
SPC 90 %	2.00	2.00	-
Soya protein	16.00	25.00	-
Corn gluten	15.00	25.00	4.00
Wheat gluten	4.00	7.30	15.00
Rapeseed cake	12.00	9.70	-
Wheat	11.08	6.80	6.00
Pea protein	-	-	13.00
Field peas	-	-	9.00
Fish oil	15.60	6.56	4.40
Rapeseed oil	0	4.40	8.80
Palm olein	0	4.40	4.80
Linseed oil	-	-	2.20
Mineral and vitamin mixtures	1.32	5.84	4.80

Table 2. Experimental conditions of the optimized UHPLC–(ESI)-MS/MS method for the selected mycotoxins.

Compound	Retention	Precursor ion		Cone	Product	Collision
Nivalenol (NIV)	0.70	$[M+H]^+$	313.1	10	175.1	20
					159.1	20
					91.0 (q2)	40
Deoxynivalenol (DON)	0.96	$[M+H]^+$	297.0	20	249.1	10
					231.1	10
					203.1	10
Fusarenon X (FusX)	1.49	$[M+H]^+$	355.1	30	175.1	20
					229.1	20
					247.1	20
Neosolaniol (NEO)	1.90	$[M+NH_4]^+$	400.2	20	185.1	20
					305.1	10
					215.1	10
3-Acetyl Deoxynivalenol (3-AcDON)	2.70	$[M+H]^+$	339.1	20	231.1	10
					213.1	20
					279.1	10
15-Acetyl Deoxynivalenol (15-AcDON)	2.78	$[M+H]^+$	339.1	20	137.0	10
					261.1	10
					297.1	10
Aflatoxin G2 (AFG2)	3.42	$[M+H]^+$	331.1	30	245.1	30
					189.1	40
					257.1	30
Aflatoxin G1 (AFG1)	3.49	$[M+H]^+$	329.1	30	243.1	30
					200.1	40
					215.1	30
Aflatoxin B2 (AFB2)	3.56	$[M+H]^+$	315.1	30	259.1	30
					287.1	30
					243.1	30
Aflatoxin B1 (AFB1)	3.62	$[M+H]^+$	313.1	30	285.1	20
					269.1	30
					241.1	30
Diacetoxyscirpenol (DIA)	3.67	$[M+NH_4]^+$	384.2	10	307.1	10
					247.1	10
					349.2	10
Fumonisin B1 (FB1)	3.85	$[M+H]^+$	722.2	30	334.2	40
					352.2	30
					686.2	30
HT-2 toxin (HT-2)	3.88	$[M+NH_4]^+$	442.2	20	263.1	10
					215.1	15
					197.0	15
T-2 toxin (T-2)	3.99	$[M+NH_4]^+$	484.2	20	185.1	20
					305.1	10
					245.1	10
Fumonisin B3 (FB3)	4.00	$[M+H]^+$	706.2	30	336.2	40
					336.2	40
					354.2	30
Fumonisin B2 (FB2)	4.04	$[M+H]^+$	706.2	30	336.2	40
					318.2	40
					354.2	30
Zearalenone (ZEN)	4.07	$[M-H]^-$	317.1	20	175.1	25
					273.1	20
					131.1	35
Ochratoxin A (OTA)	4.08	$[M+H]^+$	404.2	30	239.1	30
					221.1	35
					102.0	60

Table 3. Mean recoveries obtained from quality controls studied at two concentration levels for each group of matrices analyzed.

	INGREDIENTS (n=14)							FEED (n=4)							FILLET (n=4)					
	0.4µg/Kg			4µg/Kg				4µg/Kg			40µg/Kg				40µg/Kg			400µg/Kg		
	Rec (%)	SD	RSD	Rec (%)	SD	RSD		Rec (%)	SD	RSD	Rec (%)	SD	RSD		Rec (%)	SD	RSD	Rec (%)	SD	RSD
AFG2	74	13	17	83	14	17	AFG2	83	23	28	86	15	17	AFG2	73	27	38	74	9.7	13
AFG1	80	13	16	79	13	16	AFG1	74	4.0	5.5	85	21	24	AFG1	70	17	24	73	13	18
AFB2	82	14	17	88	14	16	AFB2	76	9.9	13	81	13	16	AFB2	67	14	22	74	7.0	9.5
AFB1	86	17	20	83	17	21	AFB1	69	1.4	2.0	80	12	16	AFB1	67	13	19	72	8.0	11
Total	81	14	18	83	14	17	Total	75	10	13	83	15	18	Total	69	18	26	73	9.5	13
FB1	46	30	65	55	25	46	FB1	80	17	21	74	17	22	FB1	68	10	15	66	8.1	12
FB2	48	20	42	62	24	39	FB2	57	15	26	59	7.4	13	FB2	66	13	20	60	4.6	7.6
FB3	53	28	53	64	23	36	FB3	57	17	30	69	3.1	4.5	FB3	65	15	24	62	5.5	9.0
T2	77	26	34	84	16	19	T2	51	7.1	14	54	10	19	T2	44	9.1	21	54	13	24
ZEN	76	22	29	71	33	47	ZEN				33	2.8	8.6	ZEN				12	2.8	24
DIA	80	12	15	87	12	14	DIA	65	12	18	78	13	17	DIA	46	12	26	70	8.9	13
NEO	93	7.9	8.5	94	8.0	8.6	NEO	83	3.5	4.2	95	13	13	NEO	70	13	19	91	7.8	8.5
Total	68	21	31	74	20	27	Total	65	12	18	66	9.4	14	Total	60	12	20	59	7	12
DON	89	34	39	70	17	24	DON	83	18	22	80	10	13	DON	81	17	21	75	9.0	12
3-AcDON	78	17	22	92	7.8	8.4	3-AcDON	93	15	16	97	10	10	3-AcDON	89	24	27	83	8.4	10
15-AcDON	71	24	34	91	11	12	15-AcDON	88	12	14	94	6.6	7.0	15-AcDON	82	19	23	85	9.0	11
HT2	81	27	33	86	19	22	HT2	64	8.1	13	67	15	22	HT2	73	20	27	64	11	17
NIV				57	23	40	NIV				57	25	45	NIV				50	0.7	1.4
Fus X	87	12	13	90	8.3	9.2	Fus X	90	17	19	92	13	14	Fus X	82	21	26	88	7.8	8.8
OTA	69	7.5	11	79	12	16	OTA	47	7.6	16	53	6.8	13	OTA	48	7.4	15	51	9.3	18
Total	82	20	25	81	14	17	Total	77	13	17	77	12	16	Total	76	18	24	71	7.9	11

Table 4. LOQs obtained for mycotoxins in the matrices studied. For two mycotoxins (nivalenol and zearalenone) no proper quantification could be obtained for some matrices due to high interference.

	LOQs (µg/Kg)																	
	Plant ingredients								Animal protein						Feed		Fish	
Mycotoxins	Wheat	Wheat Gluten	Pea	Pea Protein	Rapesed Cake	Corn Gluten	Soya Protein	Sunflower Meal	Poultry Meal	Poultry Blood Meal	Pork Meal	Pork Blood Meal	Pork greaves	Feather Meal	Salmon Feed	Sea bream Feed	Salmon	Sea bream
Nivalenol	44,4	200					182			400	400	190	364		364			
Deoxynivalenol	0,9	4,5	1,7	3,7	222	18,3	3,5	22,2	26,0	3,4	11,8	4,0	13,0	23,0	2,7	22,3	4,7	4,5
Fusarenon X	11,9	9,3	12,2	10,5	222	30,8	5,4	41,2	36,4	11,4	13,8	7,0	11,1	19,0	19,0	61,5	9,5	11,8
Neosolaniol	1,1	0,9	0,4	0,2	1,8	1,6	0,2	1,2	3,1	1,8	1,8	0,9	1,5	2,9	0,4	1,6	0,6	1,0
3 Acetyldeoxynivalenol	3,9	1,9	6,7	3,6	12,1	3,4	1,8	6,9	13,8	1,3	4,3	1,7	5,3	3,7	8,0	6,3	8,5	5,1
15 Acetyldeoxynivalenol	3,4	1,3	6,2	1,5	11,1	3,3	0,8	11,1	5,9	8,3	5,5	0,8	2,4	7,4	7,2	5,3	3,2	4,9
Aflatoxin G2	0,3	0,2	0,2	0,3	4,0	1,1	0,1	1,5	2,9	0,2	1,1	0,1	0,2	2,2	0,2	3,6	0,1	0,2
Aflatoxin G1	0,2	0,1	0,1	0,2	2,7	0,9	0,1	1,1	1,8	0,2	0,5	0,1	0,2	1,7	0,2	2,2	0,1	0,1
Aflatoxin B2	0,1	0,04	0,1	0,4	1,6	1,0	0,1	0,4	2,1	0,2	0,4	0,1	0,2	2,2	0,1	1,4	0,2	0,3
Aflatoxin B1	0,2	0,1	0,1	0,4	1,7	3,6	0,1	2,7	1,3	0,3	0,7	0,1	0,4	4,0	0,4	3,1	0,1	0,1
Diacetoxyscirpenol	0,03	0,01	0,01	0,02	0,08	0,05	0,01	0,05	0,12	0,04	0,13	0,01	0,02	0,21	0,03	0,11	0,04	0,03
HT-2 Toxin	2,4	2,1	4,8	3,7	10,9	6,9	1,6	27	14,3	5,6	11,1	2,4	4,4	30,3	4,8	30,8	3,5	5,8
Fumonisin B1	0,4	0,6	0,6	1,9	1,4	0,5	0,9	0,9	1,8	1,6	2,1	4,2	3,8	2,9	0,3	2,4	1,4	0,7
T2 Toxin	0,1	0,03	0,1	0,1	0,1	0,2	0,02	0,2	0,5	0,3	0,5	0,1	0,2	0,6	0,1	0,6	0,2	0,3
Zearalenone	0,3	14,3	1,4	0,1	4,4	0,7	4,4	8,2		6,2	5,5	5,7			36,4			
Ochratoxin A	0,4	0,3	0,4	0,7	0,7	1,4	0,2	0,5	1,6	0,9	1,4	0,6	0,8	1,7	0,6	1,5	0,8	0,9
Fumonisin B2	0,5	1,0	0,5	1,3	0,9	0,1	0,4	0,4	3,6	1,1	1,1	2,2	1,9	1,8	0,2	0,6	0,5	0,5
Fumonisin B3	0,8	1,5	0,8	1,8	3,0	0,6	1,3	0,9	7,3	2,4	1,8	4,3	4,0	6,7	1,2	3,0	1,0	1,3

Limit of detection (LOD) can be estimated from LOQ: LOD=3·LOQ/10

Table 5. Levels of mycotoxins (µg kg^{-1}ww, minimum-maximum (number of positive samples)) in commercially available processed animal proteins used in aquafeeds (n=number of different PAP samples), - = not detectable at given matrix limit in table 2 (suppl. material).

	Poultry Blood (n=4)	Poultry Meal (n=4)	Poultry Feather Meal (n=3)	Pork Blood Meal (n=3)	Pork Meal (n=3)	Pork Greaves (n=2)
AFG2	-	-	-	-	-	-
AFG1	-	-	-	-	-	-
AFB2	-	-	-	-	-	-
AFB1	-	-	-	-	-	-
NIV	-	-	-	-	-	-
Fus X	-	-	-	-	-	-
DON	-	-	-	-	-	-
3-AcDON	-	-	-	-	-	-
15-AcDON	-	-	-	-	-	-
NEO	-	-	-	-	-	-
DIA	-	-	-	-	-	-
HT-2	-	-	-	-	-	-
T-2	-	-	-	-	-	-
ZEN	-	-	-	-	-	-
OTA	-	-	-	0.4 (2)	-	-
FB1	-	0.4-2.6 (2)	0.4 (1)	-	-	-
FB2	-	-	-	-	-	-
FB3	-	-	-	-	-	-

SUPPLEMENTARY MATERIAL

Material and methods

Reagents

All mycotoxin standards (>99% purity) were supplied by Sigma Aldrich (Madrid, Spain). HPLC-grade water was obtained from water passed through a MilliQ water purification system (Millipore Ltd., Bedford, MA, USA). HPLC-grade methanol (MeOH), HPLC-grade acetonitrile (ACN), acetic acid (>99.8%) and ammonium acetate (NH_4Ac) (>99%) were purchased from ScharLab (Barcelona, Spain). Formic acid (HCOOH) (>98%) was obtained from Fluka (Buchs, Switzerland).

Mycotoxins were divided into three different groups depending on their intensity response. Thus, individual stock solutions, MIX A (10 mg L^{-1}, containing DON, HT-2, NIV, FUS X, 3AcDON, 15AcDON and OTA), MIX B (1 mgL^{-1}, containing FB1, FB2, FB3, T-2, ZEN, DIA and NEO) and MIX C (0.1 mgL^{-1}, containing AFG1, AFG2, AFB1, AFB2), respectively, were prepared by diluting the reference standards solutions in acetonitrile (abbreviations used for mycotoxins are specified in Table 1, supplementary data). The total mixed standard solution was prepared by adding 1 mL of MIX A, 1 mL of MIX B, and 1 mL of MIX C, and diluting to 10 mL with water. Working standard solutions for LC-MS/MS analysis and for fortification of samples were prepared by dilution of the total mixed standard solution with water. Stock standard solutions were stored in a freezer at -20 $^{\circ}C$, whereas working solutions were stored in a fridge. Matrix-matched standard calibration was used for quantification purposes. Standards in matrix were prepared by adding 100 µL from the corresponding standard solution plus 900 µL of the 4-fold diluted blank extract.

Analytical procedure

Matrix-matched calibration was used for a correct quantification in order to compensate for matrix effects (details in reagents, supplementary information). In every sequence of analysis (for each sample matrix), two quality control samples (QCs), i.e. "blank" samples (previously analyzed) fortified at the two different concentration levels were also analyzed together with the samples to assure reliability of data reported. QC recovery experiments were performed at 40 and 400 μg kg^{-1} for DON, HT-2, NIV, FUS X, 3-AcDON, 15-AcDON and OTA; at 4 and 40 μg kg^{-1} for fumonisins, T-2, ZEN, DIA and NEO; and at 0.4 and 4 μg kg^{-1} aflatoxins (details in reagents, supplementary information). Figure 1 shows a general overview for the QC recoveries in every matrix (ingredients, feeds and fish) for the different groups of mycotoxins. In general, recoveries (%) were satisfactory in the range between 60 and 110 %. Linearity of the quantification was evaluated taking seven matrix-matched standard solutions which were analyzed in duplicate in the following ranges: 0.01–1 ng/mL (aflatoxins), 0.1–10 ng/mL (fumonisins, T-2, ZEN, DIA, NEO) and 1–100 ng/mL (DON, HT-2 Toxin, NIV, Fus X, 3-AcDON, 15-AcDON and OTA). It was considered satisfactory when correlation coefficients were higher than 0.99 with residuals lower than 20%. Limits of quantification (LOQ) as well as limits of detection (LOD) were estimated in the matrices studied for a signal-to-noise ratio (S/N) equal to 10 and 3, respectively, from the SRM chromatograms of samples spiked at the lowest concentration level (Table 2, sup. material).

The acquisition of three SRM (Selected Reaction Monitoring) transitions per compound allowed the unequivocal confirmation of positive samples, supported by the accomplishment of ion intensity ratios and retention time when compared with reference standards. TargetLynx (MassLynx v. 4.1, Waters, Manchester, UK) software was used to process the quantitative data obtained from calibration standards and from samples. Mobile

75 phase was a time programmed gradient using A (H2O 0.1 mM NH_4Ac, 0.1% HCOOH),
76 and B (MeOH 0.1 mM NH_4Ac, 0.1% HCOOH). The percentage of organic modifier (B)
77 was changed linearly as follows: 0 min, 10%; 0.5 min, 20%; 2.5 min, 20%; 4 min, 90%;
78 4.5 min, 90%; 4.6 min, 10%; 6 min, 10% B.