

**Table 1.** Mean certificate and measured concentrations ( $\mu\text{g g}^{-1}$  in dry weight) and the associated relative standard deviation (RSD) in certified reference materials (CRM). Limit of detection (LOD) and limit of quantification (LOQ) for each element and analytical method.

Elements	Analytical method	Type	CRM		LOD ( $\mu\text{g g}^{-1}$ )	LOQ ( $\mu\text{g g}^{-1}$ )
			Certificate value ( $\mu\text{g g}^{-1}$ )	Measured value ( $\mu\text{g g}^{-1}$ )		
Hg	AAS	DORM-4	0.412 $\pm$ 0.036	0.397 $\pm$ 0.006	0.004	0.01
Cd	FAAS	DORM-2	0.065 $\pm$ 0.007	0.064 $\pm$ 0.008	0.002	0.006
Pb	FAAS	DORM-2	0.043 $\pm$ 0.008	0.042 $\pm$ 0.005	0.002	0.006
As	ICP-MS	ERM®-BB422	12.7 $\pm$ 0.7	12.0 $\pm$ 0.2	0.003	0.013
I*	ICP-MS	ERM®-BB422	1.40 $\pm$ 0.40	1.23 $\pm$ 0.02	0.01 (0.068)	0.036 (0.25)
Se	ICP-MS	ERM®-BB422	1.33 $\pm$ 0.13	1.21 $\pm$ 0.02	0.007	0.025
Cl	$\mu$ -EDXRF	SRM 1571	700	600 $\pm$ 100	100	-
K	$\mu$ -EDXRF	SRM 1571	14700 $\pm$ 300	13500 $\pm$ 1300	20	-
Ca	$\mu$ -EDXRF	SRM 1571	20900 $\pm$ 300	19500 $\pm$ 2000	30	-
Mn	$\mu$ -EDXRF	DORM-4	3.6 $\pm$ 0.3	4.0 $\pm$ 0.8	5	-
		SRM 1571	91 $\pm$ 4	88 $\pm$ 4		
Fe	$\mu$ -EDXRF	DORM-4	142 $\pm$ 10	150 $\pm$ 15	5	-
		SRM 1571	300 $\pm$ 20	298 $\pm$ 10		
Cu	$\mu$ -EDXRF	DORM-4	2.3 $\pm$ 0.2	2.4 $\pm$ 0.8	1	-
		SRM 1571	12 $\pm$ 1	13 $\pm$ 1		
Zn	$\mu$ -EDXRF	DORM-4	27 $\pm$ 2	28 $\pm$ 3	1	-
		SRM 1571	25 $\pm$ 3	24 $\pm$ 2		
Br	$\mu$ -EDXRF	SRM 1571	10	11 $\pm$ 1	1	-

\*Iodine values for fish matrix and in parentheses for feed matrix

AAS (Atomic absorption spectroscopy); FAAS (Flame atomic absorption spectrometry); ICP-MS (Inductively coupled plasma mass spectrometer); DORM-4 (Fish protein); DORM-2 (Dogfish muscle); ERM®-BB422 (Fish muscle); SRM 1571 (Orchard leaf).

**Table 2.** Growth performance of gilthead seabream and common carp from the different treatments (average  $\pm$  standard deviation).

	CTR	B1	B2	B3
<b>Gilthead seabream</b>				
IBW <sup>1</sup> (g)	371 $\pm$ 15	379 $\pm$ 2	376 $\pm$ 13	370 $\pm$ 3
FBW <sup>2</sup> (g)	626 $\pm$ 5 <sup>b</sup>	623 $\pm$ 7 <sup>b</sup>	623 $\pm$ 3 <sup>b</sup>	589 $\pm$ 5 <sup>a</sup>
TG <sup>3</sup> (%)	69 $\pm$ 5	64 $\pm$ 2	66 $\pm$ 6	59 $\pm$ 2
FCR <sup>4</sup>	1.87 $\pm$ 0.16 <sup>a</sup>	1.83 $\pm$ 0.04 <sup>a</sup>	1.90 $\pm$ 0.20 <sup>a</sup>	2.38 $\pm$ 0.35 <sup>b</sup>
SGR <sup>5</sup> (%/d)	6.22 $\pm$ 0.08	6.22 $\pm$ 0.01	6.22 $\pm$ 0.06	6.25 $\pm$ 0.03
<b>Common carp</b>				
IBW <sup>1</sup> (g)	301 $\pm$ 29	295 $\pm$ 15	295 $\pm$ 15	292 $\pm$ 20
FBW <sup>2</sup> (g)	1085 $\pm$ 16 <sup>a</sup>	1193 $\pm$ 76 <sup>b</sup>	1189 $\pm$ 50 <sup>b</sup>	1218 $\pm$ 36 <sup>b</sup>
TG <sup>3</sup> (%)	263 $\pm$ 35	306 $\pm$ 38	304 $\pm$ 12	319 $\pm$ 40
FCR <sup>4</sup>	1.52 $\pm$ 0.12	1.45 $\pm$ 0.11	1.45 $\pm$ 0.09	1.39 $\pm$ 0.11
SGR <sup>5</sup> (%/d)	1.29 $\pm$ 0.10	1.40 $\pm$ 0.10	1.40 $\pm$ 0.03	1.43 $\pm$ 0.10

Different letters (a-d) indicates significant differences between treatments (CTR – control; B1 – biofortification blend 1; B2 - biofortification blend 2; B3 - biofortification blend 3) for each species.

<sup>1</sup> Initial mean body weight

<sup>2</sup> Final mean body weight

<sup>3</sup> Total growth: (wet weight gain/IBW)  $\times$  100 .

<sup>4</sup> Feed conversion ratio: dry feed intake/wet weight gain.

<sup>5</sup> Specific growth rate:(Ln FBW– Ln IBW)  $\times$  100 / feeding days.

**Table 3.** Se:Hg molar ratio and selenium health benefit value (HBV<sub>Se</sub>) in gilthead seabream and common carp fillets from the different treatments (average  $\pm$  standard deviation).

	Se:Hg	HBV <sub>Se</sub>
<b>Gilthead seabream</b>		
CTR	4.37 $\pm$ 0.26 <sup>a</sup>	2.18 $\pm$ 0.02 <sup>a</sup>
B1	6.73 $\pm$ 0.42 <sup>b</sup>	2.89 $\pm$ 0.09 <sup>b</sup>
B2	8.19 $\pm$ 0.40 <sup>c</sup>	3.39 $\pm$ 0.10 <sup>c</sup>
B3	11.11 $\pm$ 0.83 <sup>d</sup>	4.48 $\pm$ 0.18 <sup>d</sup>
<b>Common carp</b>		
CTR	13.85 $\pm$ 1.46 <sup>b</sup>	1.18 $\pm$ 0.06
B1	9.08 $\pm$ 0.63 <sup>a</sup>	1.51 $\pm$ 0.04
B2	8.55 $\pm$ 0.18 <sup>a</sup>	1.70 $\pm$ 0.10
B3	9.52 $\pm$ 0.74 <sup>a</sup>	1.65 $\pm$ 0.13

Different letters (a-d) indicates significant differences between treatments (CTR – control; B1 – biofortification blend 1; B2 – biofortification blend 2; B3 – biofortification blend 3) for each species.

**Table 4.** Target elements percentage of the health-based guidance values (HBGVs) set by EFSA, considering the consumption of a portion of 150 g of fish fillet.

	<b>I<sup>1</sup></b>			<b>Se<sup>1</sup></b>			<b>Fe<sup>1</sup></b>			<b>Cu<sup>1</sup></b>			<b>K<sup>1</sup></b>			<b>Cl<sup>1</sup></b>		
	Adults	Children	Pregnant	Adults	Children	Pregnant	Adults	Children	Pregnant	Adults	Children	Pregnant	Adults	Children	Pregnant	Adults	Children	Pregnant
<b>Gilthead seabream</b>																		
CTR	7	7	5	39	120 (30)	32	31	118 (2)	37	19	29	20	53	156 <sup>5</sup>	47	21	26	21
B1	7	8	5	50	155 (39)	41	53	202 (4)	63	27	41	29	75	218 <sup>5</sup>	65	23	28	23
B2	8	9	6	58	181 (45)	48	41	155 (3)	48	0	0	0	67	195 <sup>5</sup>	58	17	21	17
B3	9	10	7	76	238 (59)	63	128 (10)	483 (10)	150 (10)	0	0	0	75	218 <sup>5</sup>	65	26	31	26
<b>Common carp</b>																		
CTR	2	2	1	20	62	16	65	246 (5)	76	75	114 (16)	80	39	113 <sup>5</sup>	34	4	5	4
B1	18	20	13	26	81	21	91	345 (7)	107 (7)	21	32	23	33	96	29	4	5	4
B2	21	23	16	29	91	24	92	348 (7)	108 (7)	17	25	18	39	115 <sup>5</sup>	34	5	6	5
B3	19	21	14	28	88	23	97	368 (7)	114 (7)	22	33	23	39	113 <sup>5</sup>	34	4	5	4
	<b>Ca<sup>2</sup></b>			<b>Zn<sup>2</sup></b>			<b>Hg<sup>3</sup></b>			<b>Cd<sup>3</sup></b>			<b>Pb<sup>4</sup></b>					
	Adults	Children	Pregnant	Adults	Children	Pregnant	Adults	Children	Pregnant	Adults	Children	Pregnant	Adults	Children	Pregnant			
<b>Gilthead seabream</b>																		
CTR	14	18	14	2	3	2	6	20	6	1	5	1	30	106 (22)	31			
B1	7	9	7	4	4	3	5	17	5	1	3	1	32	114 (24)	33			
B2	4	5	4	2	3	2	5	16	5	1	4	1	31	112 (23)	33			
B3	2	3	2	2	2	2	4	16	5	2	6	2	23	82	24			
<b>Common carp</b>																		
CTR	25	32	25	28	32	20	1	3	1	1	3	1	35	125 (26)	36			
B1	8	10	8	33	38	24	2	7	2	1	3	1	22	77	23			
B2	19	24	19	33	38	24	2	8	2	1	3	1	26	92	27			
B3	8	10	8	33	38	24	2	7	2	1	4	1	26	95	28			

<sup>1</sup> Percentages were calculated according to the adequate intakes (AI) as well as the tolerable upper intake level (UL; in parenthesis) set by EFSA (2014b, 2014c, 2015a, 2015b, 2015c, 2016, 2019). <sup>2</sup> Percentages were calculated according to the adequate requirement (AR) set by EFSA (2015d, 2014e); <sup>3</sup> Percentages were calculated according to the tolerable weekly intake (TWI) set by EFSA (2012a, 2011); <sup>4</sup> Percentages were calculated according to the benchmark dose lower limit (BMDL<sub>01</sub>) as well as the margin of exposure (MOE; in parenthesis) set by EFSA (2010). Data was calculated using adults (> 18 years), children (1-3 years) and pregnant/lactating women's (18-35 years) mean body weights in Europe (body weight: 70, 13 and 67 kg, respectively; EFSA, 2012b). CTR – Control treatment; B1 – treatment B1; B2 - treatment B2; B3 - treatment B3. <sup>5</sup>No tolerable upper intake level (UL) has been set for potassium by EFSA due to insufficient data (EFSA, 2016a).

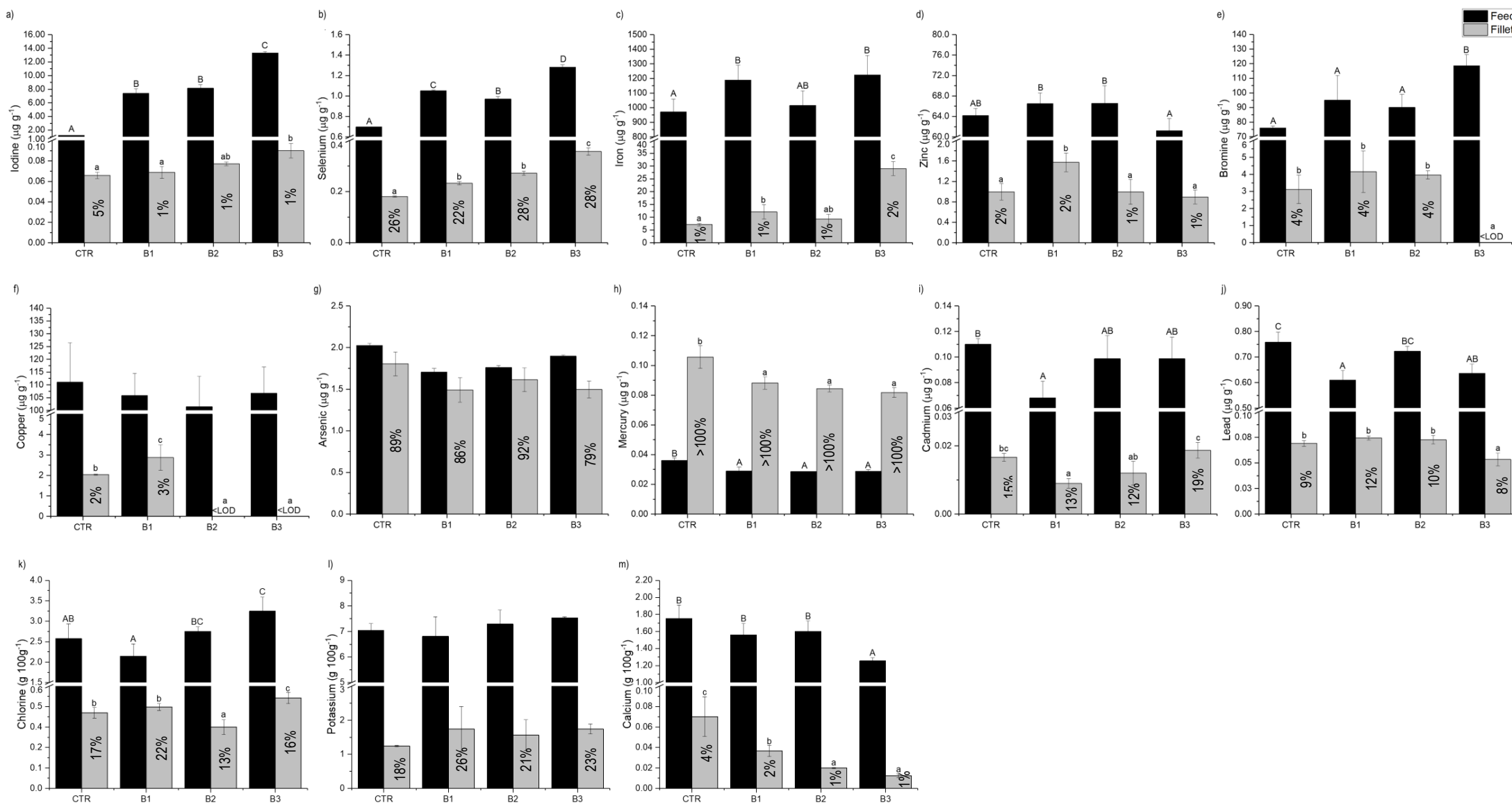


Figure 2. Levels of trace elements (a - Iodine, b - Selenium, c - Iron, d - Zinc, e - Bromide, f - Copper; in  $\mu\text{g g}^{-1}$ ), toxic elements (g - Arsenic, h - Mercury, i - Cadmium, j - lead; in  $\mu\text{g g}^{-1}$ ) and macro elements (k - Chlorine, l - Potassium, m - Calcium; in  $\text{g } 100\text{g}^{-1}$ ) in gilthead seabream diets and fillets (average  $\pm$  SD, in wet weight); and percentages of element deposition in fish fillet from each diet. Different capital letters (A - D) represents significant differences ( $p < 0.05$ ) in elements concentration between diets (CTR - control, B1 - biofortified B1, B2 - biofortified B2, B3 - biofortified B3), whereas small letters (a - d) represents significant differences ( $p < 0.05$ ) between fillets (CTR, B1, B2, B3).

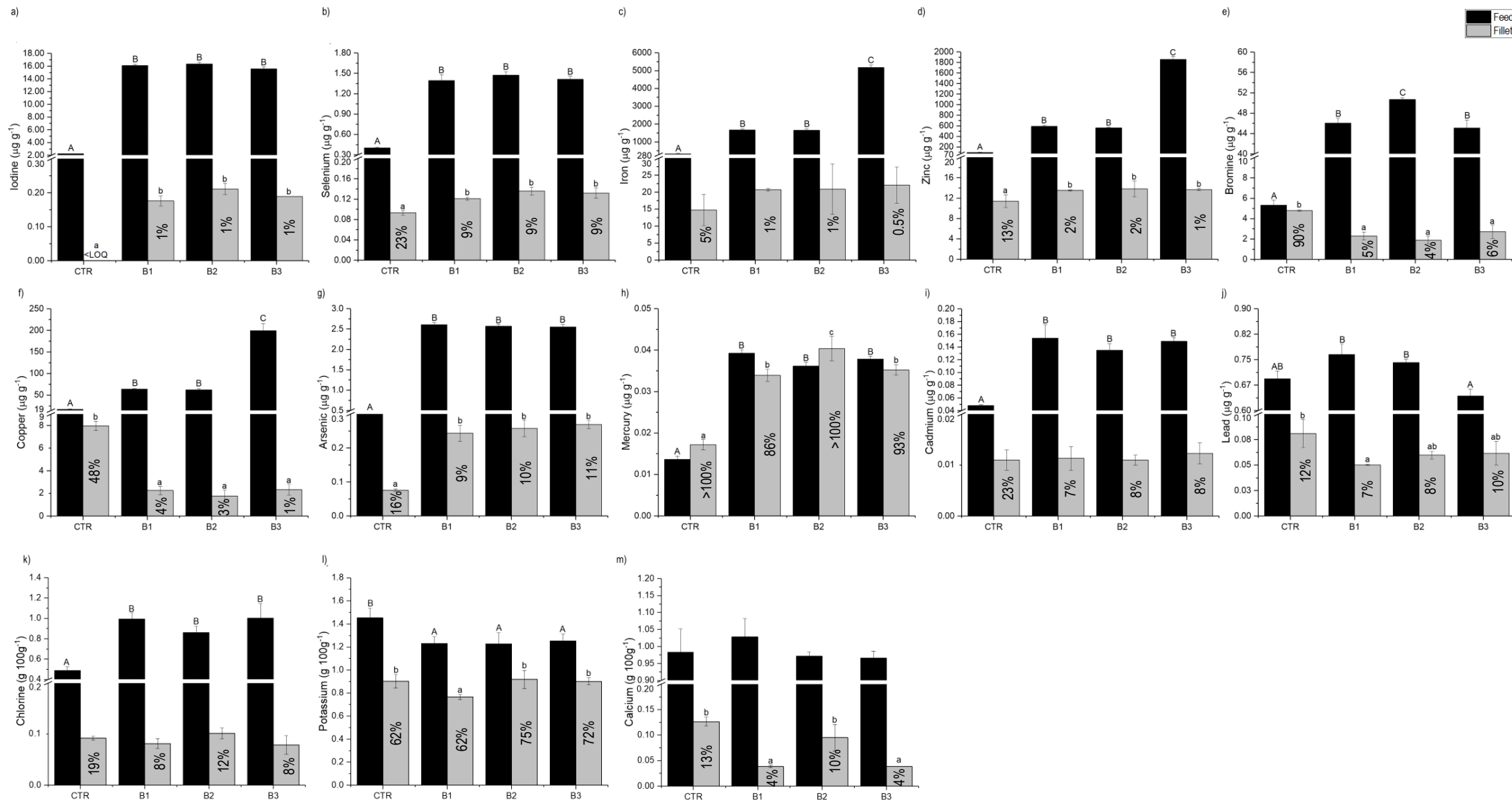


Figure 3. Levels of trace elements (a - Iodine, b - Selenium, c - Iron, d - Zinc, e - Bromide, f - Copper; in  $\mu\text{g g}^{-1}$ ), toxic elements (g - Arsenic, h - Mercury, i - Cadmium, j - Lead; in  $\mu\text{g g}^{-1}$ ) and macro elements (k - Chlorine, l - Potassium, m - Calcium; in  $\text{g } 100\text{g}^{-1}$ ) in common carp diets and fillets (average  $\pm$  SD, in wet weight); and percentages of element deposition in fish fillet from each diet. Different capital letters (A-D) represents significant differences ( $p < 0.05$ ) in elements concentration between feed (CTR- control, B1 - biofortified B1, B2 - biofortified B2, B3 - biofortified B3), whereas small letters (a-d) represents significant differences ( $p < 0.05$ ) between fillets (CTR, B1, B2, B3).

**Table S3.** Gilthead seabream (*S. aurata*) and common carp (*C. carpio*) initial (Baseline) and final (in each treatment: CTR, B1, B2, B3) total length (cm) and weight (g)

Species	Treatment	n	Total length (cm)	Total weight (g)	Moisture (%)
Gilthead seabream	Baseline	15	31 ± 2 (28 - 34)	491 ± 68 (380 – 584)	69 ± 3.9
	CTR	15	33 ± 1 (31 - 36)	578 ± 70 (483 – 692)	69 ± 0.6
	B1	15	32 ± 1 (30 - 35)	531 ± 74 (427 – 664)	68 ± 0.5
	B2	15	33 ± 2 (30 - 35)	574 ± 70 (460 – 677)	69 ± 1.1
	B3	15	33 ± 2 (30 - 36)	578 ± 62 (463 – 666)	69 ± 0.9
Common carp	Baseline	15	29 ± 3(26 – 37)	333 ± 44 (250 – 400)	78 ± 0.3
	CTR	15	40 ± 2 (37 – 43)	1236 ± 108 (1027 – 1443)	78 ± 1.0
	B1	15	41 ± 1 (40 – 42)	1226 ± 106 (1095 – 1397)	78 ± 0.3
	B2	15	40 ± 2 (37 – 42)	1217 ± 105 (1045 – 1440)	77 ± 1.3
	B3	15	41 ± 2 (37 – 43)	1338 ± 112 (1133 – 1493)	78 ± 0.7

Treatment, baseline (initial) and at the end of the feeding trial (final) in control diet (CTR) and three different fortified diets (B1 - biofortified B1, B2 - biofortified B2, B3 - biofortified B3); n, number of specimens analysed; total length (cm) and total weight (g) – mean ± SD (range minimum and maximum).

**Table S5.** Gilthead seabream (*S. aurata*) and common carp (*C. carpio*) fillets initial (Baseline) and final (CTR) elemental composition.

	I (mg g <sup>-1</sup> )	Se (mg g <sup>-1</sup> )	Fe (mg g <sup>-1</sup> )	Zn (mg g <sup>-1</sup> )	Br (mg g <sup>-1</sup> )	Cu (mg g <sup>-1</sup> )	As (mg g <sup>-1</sup> )	Hg (mg g <sup>-1</sup> )	Cd (mg g <sup>-1</sup> )	Pb (mg g <sup>-1</sup> )	Cl (g 100g <sup>-1</sup> )	K (g 100g <sup>-1</sup> )	Ca (g 100g <sup>-1</sup> )
<b>Gilthead seabream</b>													
Baseline	0.06 ± 0.004	0.18 ± 0.01	7.4 ± 1.6	0.9 ± 0.1	3.3 ± 0.2	2.4 ± 0.04	1.8 ± 0.06	0.1 ± 0.006	0.01 ± 0.002	0.06 ± 0.001	0.2 ± 0.01 <sup>a</sup>	1.7 ± 0.3	0.07 ± 0.004
CTR	0.07 ± 0.003	0.18 ± 0.002	7.1 ± 0.5	1.0 ± 0.1	3.1 ± 0.4	2.0 ± 0.02	1.8 ± 0.1	0.1 ± 0.008	0.02 ± 0.001	0.07 ± 0.003	0.4 ± 0.03 <sup>b</sup>	1.2 ± 0.02	0.07 ± 0.02
<b>Common carp</b>													
Baseline	0.01 ± 0.001	0.07 ± 0.002 <sup>a</sup>	10 ± 1.5	9 ± 0.4 <sup>a</sup>	1.1 ± 0.3 <sup>a</sup>	1.3 ± 0.1 <sup>a</sup>	0.03 ± 0.002 <sup>a</sup>	< LOQ <sup>a</sup>	0.01 ± 0.002	0.08 ± 0.01	0.09 ± 0.03	0.8 ± 0.01	0.04 ± 0.0005 <sup>a</sup>
CTR	0.02 ± 0.001	0.09 ± 0.005 <sup>b</sup>	15 ± 1.6	11 ± 1.2 <sup>b</sup>	4.8 ± 0.1 <sup>b</sup>	8 ± 0.4 <sup>b</sup>	0.08 ± 0.004 <sup>b</sup>	0.02 ± 0.001 <sup>b</sup>	0.01 ± 0.002	0.08 ± 0.02	0.09 ± 0.004	0.9 ± 0.06	0.13 ± 0.01 <sup>b</sup>

Different letters (a, b) represents significant differences ( $p < 0.05$ ) between fish fillets from baseline (initial) and in the final of the experimental feeding trial with control (CTR) diet. Values are average ± standard deviation in wet weight.