

1 **Occurrence and potential transfer of mycotoxins in gilthead sea bream and**
2 **Atlantic salmon by use of novel alternative feed ingredients.**

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28 **Abstract**

29 Plant ingredients and processed animal proteins (PAP) are suitable alternative feedstuffs for
30 fish feeds in aquaculture practice, although their use can introduce contaminants that are not
31 previously associated with marine salmon and gilthead sea bream farming. Mycotoxins are
32 well known natural contaminants in plant feed material, although they also could be present
33 on PAPs after fungi growth during storage. The present study surveyed commercially
34 available plant ingredients (19) and PAP (19) for a wide range of mycotoxins (18) according
35 to the EU regulations. PAP showed only minor levels of ochratoxin A and fumonisin B1 and
36 the mycotoxin carry-over from feeds to fillets of farmed Atlantic salmon and gilthead sea
37 bream (two main species of European aquaculture) was performed with plant ingredient
38 based diets. Deoxynivalenol was the most prevalent mycotoxin in wheat, wheat gluten and
39 corn gluten cereals with levels ranging from 17 to 814 and $\mu\text{g kg}^{-1}$, followed by fumonisins
40 in corn products (range 11.1-4901 $\mu\text{g kg}^{-1}$ for fumonisin B1+B2+B3). Overall mycotoxin
41 levels in fish feeds reflected the feed ingredient composition and the level of contaminant in
42 each feed ingredient. In all cases the studied ingredients and feeds showed levels of
43 mycotoxins below maximum residue limits established by the Commission Recommendation
44 2006/576/EC. Following these guidelines no mycotoxin carry-over was found from feeds to
45 edible fillets of salmonids and a typically marine fish, such as gilthead sea bream. As far we
46 know, this is the first report of mycotoxin surveillance in farmed fish species.

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48 **Keywords:** Mycotoxins, marine aquaculture, plant ingredients, processed animal
49 proteins, fish feed, fish

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53 **1. Introduction**

54 Serious concern on fish meal and fish oil availability to support the rapidly growing
55 aquaculture industry has led to extensive search of alternative raw materials for
56 aquafeeds (Tacon and Metian, 2008; Torrissen et al., 2011). The most obvious
57 alternatives are plant oils and proteins, and the long-term consequences of high
58 inclusion levels of these feedstuff have been addressed in past and ongoing large EU
59 projects, such as AQUAMAX (www.aquamaxip.eu) and ARRAINA (www.rraina.eu),
60 where main results highly support the feasibility of a high level of replacement of
61 marine feed ingredients in both Atlantic salmon (*Salmo salar*) and gilthead sea bream
62 (*Sparus aurata*) (Benedito-Palos et al., 2008; Torstensen et al., 2008). Processed animal
63 protein (PAP) from the rendering industry is another valuable alternative feed ingredient
64 (Davies et al., 2009; Burr et al., 2012; Toldra et al., 2012), and recently the EU has set
65 out a working plan for the re-authorization of the use of non-ruminant PAPs in
66 aquafeeds after previous bans following outbreaks of transmissible spongiform
67 encephalopathies (EC, 2013a).

68 The use of these alternative feed ingredients can introduce contaminants that
69 were previously not associated with marine salmon and sea bream farming. One
70 example of this are mycotoxins, which are world-wide found in cereal grains and animal
71 feed (Binder, 2007 a,b; Beltran et al, 2013; Streit et al., 2013). Mycotoxins are produced
72 by fungi that pre-harvest infect agricultural crops (field mycotoxins) or post-harvest
73 agricultural commodities stored under certain temperature and humidity conditions
74 (storage mycotoxins) (Magan et al., 2010; Bryden, 2012). Meat products can also be
75 contaminated with mycotoxins (Mizáková et al., 2002; Sorensen et al., 2010; Ostry et
76 al., 2013), and animal by-products could hence be a potential source for these
77 mycotoxins in animal feeds (Caruso et al., 2013). The mycotoxin aflatoxin B1 (AFB1)

78 is under EU feed regulation (EU, 2002), while guidance values have been set for animal
79 feed ingredients and animal feed for several mycotoxins, including deoxynivalenol
80 (DON), zearalenone (ZEN), ochratoxin A (OTA), and fumonisin B1 + B2
81 (FB1+FB2)(EC, 2006). For other mycotoxins, such as T-2 and HT-2 toxins, indicative
82 levels for cereal products, including those intended for animal feed have been set (EC,
83 2013b; Cheli et al., 2014). In fact, many surveillance studies have reported mycotoxin
84 levels on a wide range of randomly sampled feed ingredients and finished feeds from
85 terrestrial animals (Binder, 2007 a,b; Rodrigues and Naehrer, 2012; Streit et al., 2012;
86 Streit et al., 2013), but only few studies recent studies are done in fish feeds or farmed
87 fish (Pietsch et al., 2013; Wozny et al., 2013). Besides, most fish studies on mycotoxins
88 are focused on the hazards for fish health in experimental trials with fortified feeds
89 (Poston et al., 1982; Arukwe et al., 1999; Manning et al., 2003; EFSA, 2005; Manning
90 et al., 2005; Wozny et al., 2008; EFSA, 2011; Hoofst et al., 2011; Caruso et al., 2013)
91 with little information on the carry-over to the edible parts of the fish.

92 Multi occurrence of mycotoxins requires, however, the need for the application
93 of multi-mycotoxin methods in order to get a more accurate picture of the extent of the
94 wide range of mycotoxin contamination (Beltran et al., 2009, 2013; Monbaliu et al.,
95 2010; Streit et al., 2012; Aberg et al., 2013). Earlier studies established feasible
96 analytical approaches for mycotoxins in feed ingredients, aquafeeds and fish fillets
97 (Malachová et al., 2014; Beltran et al., 2013; Nacher-Mestre et al., 2013). Based on this
98 previous experience, the present work aims to quantify a wide range of mycotoxins in
99 commercially available plant and PAP feed ingredients, fish feeds based on these
100 ingredients, and their transfer to the edible part of farmed Atlantic salmon and gilthead
101 sea bream, two main species of the European aquaculture. In addition to the 8
102 mycotoxin under EU regulation/guidance in feed and feed ingredients (AFB1, DON,

103 ZEN, OTA, FB1+FB2, T-2 and HT-2), 10 additional mycotoxins of potential relevance
104 for food safety are included (AFB2, AFG1, AFG2, FB3, nivalenol (NIV), 3-
105 acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON),
106 diacetoxyscirpenol (DIA), fusarenon-X (Fus X) and neosolaniol (NEO)) in the study.

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108 **2. Material and methods**

109 *2.1. Feed ingredients*

110 A total of 19 commercially available plant feed ingredients were provided by Biomar
111 (Grangemouth, UK) feed producer: wheat (n=3, Germany and Denmark), wheat gluten
112 (n=4, UK, Germany, and China), pea (n=1, Denmark), pea protein (n=2, Norway),
113 rapeseed meal (n=1, Denmark), corn gluten (n=3, China and Germany), soya protein
114 (n=4, Brazil) and sunflower meal (n=1, Russia). Nineteen commercially available PAPs
115 from non-ruminants were provided by the European Fat Processors and Renderers
116 Association (EFPPRA). All PAPs were produced according the EU regulation for PAP
117 intended for use as feed-ingredients in animal feed (EC, 2001, 2009). These PAPs are
118 category 3 products that are fit for human consumption at the point of slaughter (EC,
119 2009). The PAPs sourced are all produced in central Europe and included poultry bone
120 and meat meal (n=4), poultry blood meal (n=4), pork meal (n=3), pork blood meal
121 (n=3), pork greaves (n=2) and feather meal (n=3). All feed ingredients were stored at -
122 18° C until analyses.

123

124 *2.2. Experimental diets and feeding trials*

125 Fish feeds for feeding trials were based on plant feed ingredients, and not PAPs, as only
126 noticeable mycotoxin levels were found on the former feedstuffs (see results section).
127 The feeds were produced by Biomar under commercial aquafeed production techniques

128 based on high-temperature extrusion processes, which potentially could affect
129 mycotoxin residue levels. For gilthead sea bream, two diets were formulated with the
130 same feed ingredients varying the replacement of fish meal and fish oil by plant
131 ingredients. Salmon feeds were production triplicates of high plant ingredient diets
132 based on the same feed ingredients (Table 1, sup. data).

133 Sea bream trial. Juvenile gilthead sea bream of Atlantic origin were fed with the
134 respective diet (triplicate tanks of 2500 L in groups of 150 fish each) for 8 months
135 (May-December) in the indoor experimental facilities of the Institute of Aquaculture of
136 Torre la Sal (CSIC, Spain) under natural light and temperature conditions at our latitude
137 (40°5'N; 0°10'E). Fish grew from an initial body weight of 15 g until 296-320 g with a
138 feed:gain ratio (feed/weight gain) of 1-1.05 regardless of diet composition. Over the
139 course of the trial, fish were fed daily (5-6 days per week) at visual satiety. At harvest
140 (week 31), 6 fish per dietary treatment were killed by a blood to the head and deboned
141 fillets were stored at -80 °C until analyses.

142 Salmon trial. Post-smolts were randomly distributed among 6 sea cages (5m x 5m x 5m;
143 125 m³; 150 fish per cage) at Gildeskål Research Station, GIFAS, Gildeskål kommune,
144 Norway. Prior to the start of the trial, fish were acclimated to the environmental
145 conditions for two weeks. At the start, the average fish weight was 228 ± 5 g and during
146 the 6th month feeding period (duplicate cages per diet) the weight fish is more than
147 doubled. Over the course of the trial, fish were hand-fed until satiation two times daily
148 and feed intake was recorded for each sea cage. At harvest (week 27), 3 fish per dietary
149 treatment were killed by a blood to the head and deboned fillets were stored at -80 °C
150 until analyses.

151

152 2.3. Analytical procedure

153 Up to 18 mycotoxins, AFB1, AFB2, AFG1, AFG2, OTA, NEO, FB1, FB2, FB3, T-2,
154 DIA, ZEN, NIV, DON, 3-AcDON, 15-AcDON, Fus X, and HT-2 were analyzed
155 according to the methodology of Beltran et al. (2013), adapted to the aquaculture
156 matrices (Nacher-Mestre et al., 2013). Briefly, 2.5 g homogenized samples were
157 extracted with acetonitrile:water 80:20 (1% HCOOH) using an automatic mechanical
158 shaker for 90 min. Then, the extract was centrifuged followed by a 4-fold dilution with
159 water and finally centrifuged prior analysis. Analyses were performed by ultra-high
160 performance liquid chromatography (UHPLC, BEH C18 analytical column, 1.7 µm
161 particle size, 2.1 mm × 50 mm; Acquity, Waters, Milford, MA, USA,) coupled to
162 tandem mass spectrometry (MS/MS) with a triple quadrupole analyser (QqQ; TQ-S,
163 Waters Micromass, Manchester, UK) using an orthogonal Z-spray-electrospray
164 interface (ESI). More details for LC-MS/MS conditions (table 2, sup. data), reagents
165 and analytical procedure (Material and methods, sup. data) could be consulted in
166 supplementary material.

167

168 **3. Results and Discussion**

169 The multi mycotoxin LC-ESI-MS/MS method was applied to the analysis of 18
170 mycotoxins in plant and animal ingredients used in the elaboration of fish feed, in
171 different experimental feeds and in cultured fish tissues from marine aquaculture trials.
172 Results of the QC recoveries included in each batch were in the range between 60 and
173 110% with few exceptions for fish fillet matrices (Table 3, supplementary material).
174 Figure 1 shows a general overview for the QC recoveries in every matrix (ingredients,
175 feeds and fish) for the different groups of mycotoxins (more details related to recovery
176 values in Table 3, supplementary material). Regarding matrix effects, fumonisins, DON,
177 OTA and ZEN were the compounds which showed higher matrix suppression in all

178 matrices studied. LOQs at concentrations around the level of $\mu\text{g kg}^{-1}$ were obtained for
179 almost all studied mycotoxins (Table 4, supplementary material). For two mycotoxins
180 no proper quantification could be obtained for some matrices (NIV in rapeseed, corn,
181 pea, poultry feather and blood meal and ZEN in poultry feather and blood meal and
182 pork meal) due to the presence of coeluted matrix interference peaks. The LOQs for the
183 different ingredients, feeds and fish muscle from the feeding experiments were in all
184 cases below the maximum permitted levels (EU, 2002; EC, 2006, 2013b).

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186 3.1. Feed ingredients of plant origin

187 Table 1 gives the level of mycotoxins in plant feed ingredients that are
188 commonly used in commercial aquafeeds for Atlantic salmon and gilthead sea bream.
189 Fumonisin (sum FB1+FB2+FB3) in corn was the most prevalent mycotoxin
190 contamination (min.-max. 11.1-4901 $\mu\text{g kg}^{-1}$) followed by DON in wheat and corn
191 products (min-max. 17-504 and 139-814 $\mu\text{g kg}^{-1}$, respectively). Fumonisin were also
192 present in one wheat gluten sample, but with lower levels (13.2 $\mu\text{g kg}^{-1}$) than observed
193 in corn. As fumonisin contamination of wheat is not common, possible contamination
194 from contaminated corn cannot be excluded. ZEN as well as T-2 and HT-2 were found
195 in some of the wheat and corn feed ingredients (min-max. 8-17 and 2.8-67 $\mu\text{g kg}^{-1}$,
196 respectively). OTA was found in wheat, corn and pea protein products (min-max. 0.4-
197 5.2 $\mu\text{g kg}^{-1}$ for all products). All levels were under the EU regulation or guidance levels
198 for mycotoxins in plant material intended for animal feeds (Cheli et al., 2014).

199 Plant feed ingredients for aquafeeds are sourced from the global market and in
200 the present study ingredients were obtained from Asia, South-America and central and
201 Northern Europe. The current study included only a limited number of possible plant
202 feed ingredients used for aquafeeds, not providing a basis for global mycotoxin

203 contamination assessment. Other studies on plant feed ingredients used for terrestrial
204 animal feeds, however, have performed a far more extensive global surveillance
205 showing regional and plant specific differences in mycotoxin contamination (Binder et
206 al., 2007b; Monbaliu et al., 2012; Njobeh et al., 2012; Rodrigues and Naehrer, 2012;
207 Afsah-Hejri et al., 2013; Schatzmayr and Streit, 2013; Streit et al., 2013).

208 In Northern world-wide regions such as North-America, North-Asia and central
209 Europe the main corn contaminants are DON (average levels ranging 1085-1421 $\mu\text{g kg}^{-1}$)
210 and fumonisins (average levels ranging 1357-2861, 2180 $\mu\text{g kg}^{-1}$). In contrast, in
211 Southern regions such as South-America, South-East Asia and Southern Europe the
212 corn has far lower DON than fumonisins levels (average levels ranging 214-985 and
213 1568-3226, $\mu\text{g kg}^{-1}$, respectively) (Rodrigues and Naehrer, 2012). Similarly in the
214 present study, one corn sample from South-China had a lower DON than fumonisins
215 level (815 versus 4901, $\mu\text{g kg}^{-1}$ respectively) while the other two corn samples from
216 Europe (Germany) had lower and more equal DON and fumonisin levels. Both central-
217 European corn samples also had relatively high trichothecenes levels such as HT-2
218 toxin (67 $\mu\text{g kg}^{-1}$) followed by ZEN (8 $\mu\text{g kg}^{-1}$), as could be expected for *fusarium* fungi
219 producing toxicants in moderate climates (Binder et al. 2007b). The *fusarium* fungi
220 species are the most common source for corn fumonisins contamination but also
221 *Aspergillus niger* produces fumonisins on corn, mainly as FB2 (Soares et al., 2013).
222 Corn is a plant feed ingredient that is most affected by co-contamination of several
223 mycotoxins (Scudamore and Livesey, 1998) and similarly in the present study the corn
224 samples had co-occurrence of fumonisins B1, B2 and B3, DON, 15-AcDON, HT-2, T-
225 2, ZEN, and OTA.

226 Earlier global surveillance showed that DON was the main wheat contaminant
227 independently from region of origin (Rodrigues and Naehrer, 2012). Similarly, for

228 wheat products in the present trial which were sourced from central Europe and Asia,
229 DON was the main contaminant followed by ZEN and to a lesser degree T-2, HT-2
230 toxin and fumonisins (Table 1). Soybean meal products are widely used feed ingredient
231 in Atlantic salmon and sea bream farming, and only GMO-free soy products are used
232 which are mostly source from Brasil. Global surveys showed DON and fumonisins
233 equally present in soy from South-America, but at far lower levels than wheat and corn
234 (Rodrigues and Naehrer, 2012). In present study soy had only low mycotoxin
235 contaminations compared to wheat and especially corn (Table 1).

236 The mycotoxin OTA is mostly produced by *penicillium* species under storage
237 conditions and was mainly found in the present study in wheat and pea proteins (Table
238 1). The fungi *P. verrucosum* is typically primarily found on cereals and is therefore
239 responsible for the major contributor to OTA contamination of cereal products (Lund
240 and Frisvad, 2003). OTA can also be produced by several *Aspergillus* species which are
241 adapted to grown on various leguminous seeds (Bayman et al., 2006) which could
242 explain the low OTA contamination of peas. Clearly, as for terrestrial animal farming,
243 sourcing of plant feed ingredients based on product type and regions of origin is a first
244 step in control of mycotoxin aquafeed contamination.

245

246 3.2. Feed ingredients of animal origin

247 As expected, only the typical storage mycotoxins, OTA (at concentrations below 0.4 μg
248 kg^{-1}) but also FB1 (at concentrations between 0.4-2.6 μg kg^{-1}), were detected in poultry
249 feather and bone and meat meal (fumonisin) and pork blood (OTA), respectively (Table
250 5, supplementary material). The levels were, however, around detection limit and are by
251 far under the EU guidelines for plant products intended for animal feeds (60 mg kg^{-1} for
252 FB1+FB2 in maize and 250 μg kg^{-1} for OTA in cereals (EC, 2006)). Fumonisin are

253 mainly produced by a small number of *Fusarium* species, which have specific crops
254 (corn) as habitat (Pitt and Hocking, 2009). However, other fumonisin producing fungi
255 such as *Aspergillus niger* (Mogensen et al., 2009) has been isolated from warm air-dried
256 meat products (Mizáková et al., 2002; Sorensen et al., 2010). The most common
257 fumonisin produced by *Aspergillus niger* is FB2 at high amounts of carbohydrate or
258 NaCl (Frisvad et al., 2007), although additional FB4 can be produced in agar cultures
259 (Noonim et al., 2009) and other fumonisins forms (FB1-4) are found on *A. niger*
260 contaminated dried raisins (Varga et al., 2010). In the present study, FB1 was the only
261 fumonisin form detected on PAP material albeit at low levels. The absence of FB2-3
262 might be due to the LOQs, which were higher than for FB1 in PAP material such as
263 poultry meal. The *Aspergillus niger* strains are also known to produce OTA (Accensi et
264 al., 2004), which could be a source for the detected OTA in one of the PAP samples.
265 The fungi *Penicillium nordicum* is the most known OTA producer (Larsen et al., 2001;
266 Lund and Frisvad, 2003) and grows well at low temperatures on meat products but
267 mostly only at increased salinity (Schmidt-Heydt et al., 2012). Storage OTA
268 contamination by *P. nordicum* is, therefore, often limited to salted meat food products
269 such as cured ham and sausage (Sonjak et al., 2011; Schmidt-Heydt et al., 2012).
270 Products of animal origin such as pork and poultry raw meat or blood products can be
271 also indirectly contaminated by OTA when monogastric animals are fed with
272 contaminated feed stuffs (EFSA, 2004a) as dietary OTA can be transferred from the
273 feed to animal meat (Malagutti et al., 2005). From the present study, however, the risk
274 of OTA or FB1+B2+B3 contamination of EU produced PAP products intended for
275 aquafeeds seems low. Similarly, from surveillance studies on foodstuffs of both plant
276 and animal origin it was concluded that plant products rather than cured animal products
277 could be contaminated with OTA (Bertuzzi et al., 2013; Ostry et al., 2013)

278

279 3.3. Feed and fish muscle

280 The present study assess the transfer of mycotoxins throughout the sea bream
281 and Atlantic salmon food production chain by assessing mycotoxin levels in feed
282 ingredients and follow their transfer to commercially produced aquafeeds and
283 eventually carry-over to the edible parts of the fish fed on these feeds. Commercially
284 produced aquafeeds were made based on the same analysed plant feed ingredients given
285 in table 1. Table 2 gives the mycotoxin levels of sea bream feed with either a low or
286 high overall plant protein content as well as three Atlantic salmon feed production
287 repeats with a similar high-plant feed composition based on the same batch of feed
288 ingredients. For the sea bream feeds, the low plant-protein feeds had an unexpected
289 higher DON level than high-plant protein based feeds. One of the main sources for
290 DON in sea bream diets was contaminated wheat ($371 \mu\text{g kg}^{-1}$), which inclusion levels
291 in low plant feed was slightly higher than high plant feed (11 versus 7 %, respectively),
292 thus explaining the slightly higher levels in low-plant diets. The wheat gluten used in
293 the sea bream diets had only minor DON levels ($17 \mu\text{g kg}^{-1}$). For the sea bream feeds,
294 the main plant-protein increase in high plant protein diets came from corn and soya
295 (from 31% to 50 % : low plant feed with 15% and 16% and high plant feed with 25%
296 and 25% for corn and soya, respectively). The soy protein concentrate (SPC) feed
297 ingredient had only detectable levels of FB1 and FB2, while corn was the main source
298 for fumonisin ($139 \mu\text{g kg}^{-1}$ sum FB1+2+3) and 15 Ac-DON ($53 \mu\text{g kg}^{-1}$), causing an
299 increase in these mycotoxins in high plant-protein feeds. The differences in mycotoxin
300 contamination of traditional marine feeds and high plant-protein substitution feeds in
301 the present study, exemplifies that mycotoxin levels in plant-protein based feeds are
302 more dependent on the individual contamination level of each plant-protein ingredient

303 rather than the overall higher inclusion level of plant protein. In addition to the
304 substitution of fish-meal with plant-proteins, an extra sea bream feed was produced in
305 which fish oil was substituted with plant oils. This substitution had no effect on feed
306 mycotoxin level supporting the notion that the plant proteins and not the plant oils are
307 the main source for mycotoxin contamination.

308 For the Atlantic salmon high plant-protein feed production repeats, mycotoxin
309 levels were as expected from the contamination level of the feed ingredients and with
310 similar levels among the repeats with the exception of fumonisins. Higher feed
311 fumonisin levels were found than could be expected from the low inclusion level (4%)
312 of the sole fumonisin feed ingredient source (corn, 403 $\mu\text{g kg}^{-1}$ sum FB1+2+3), and with
313 a large variation (112-754 $\mu\text{g kg}^{-1}$ sum FB1+2+3) among the production repeats. The
314 large variation in fumonisin levels suggest the present of storage fungi that can grow
315 heterogeneously within and among feed batches. The main source for fumonisins in
316 corn are *Fusarium* species which normally grow very little under storage conditions and
317 storage is not expected to increase *furasiium* derived fumonisin contamination (Pitt et
318 al., 2013). *Aspergilles niger* fungi species can also produce fumonisins (Baker, 2006)
319 but they are also the source for the typical storage mycotoxin OTA, which was were
320 only present at detectable levels in the salmon feeds as could be excepted from the
321 inclusion of OTA contaminated pea proteins (1.8 $\mu\text{g kg}^{-1}$ at inclusion level of 13%).
322 Surveillance of finished feed for terrestrial animals in Europe and the Mediterranean
323 area gave average fumonisin levels of 638 $\mu\text{g kg}^{-1}$ in 3 out of 10 analysed samples
324 (Binder, 2007b). Slovenian poultry feed had fumonisin levels ranging from 36-1160 μg
325 kg^{-1} (Streit et al., 2012). Surveillance of feed ingredients and finished feeds in Europe
326 and the Mediterranean showed maximum OTA level in feed ingredients to be 33 $\mu\text{g kg}^{-1}$
327 while in finished feeds the mean levels were 305 $\mu\text{g kg}^{-1}$ with maximum of 530 $\mu\text{g kg}^{-1}$,

328 thus suggesting OTA contamination during storage of finished feeds. Studies on
329 rainbow trout feeds in Poland showed ZEN contamination up to 82 $\mu\text{g kg}^{-1}$; in the
330 present study however ZEN was not detected in any of the feeds.

331 Information on carry-over of contaminants from feed ingredients and feed to animal
332 food products is essential for appropriate human risk assessment of feed contaminants
333 (Leeman et al., 2007). Expert opinions by the European Food Safety Authorities (EFSA)
334 have evaluated the carry-over of several mycotoxins in terrestrial animals such as poultry,
335 swine and cow (EFSA, 2004a,b, 2005, 2007), while no information exists on the carry-over
336 in farmed fish species. In the present study, neither gilthead sea bream nor Atlantic salmon
337 had any detectable levels of mycotoxins in their fillet (data not shown) after respectively 8
338 and 7 months of feeding with the diets presented in table 2. In general, the carry-over of
339 mycotoxins in terrestrial animals is limited (EC, 2006) which is partly the basis for the use of
340 only guidance limits and not regulation limits for mycotoxins in feeds (with the exception of
341 the aflatoxins) as contaminated feed does not directly or indirectly impact the human health
342 (Siegel and Babuscio, 2011). Similarly in the present study, for marine farmed sea bream and
343 Atlantic salmon, the potential carry-over of mycotoxin residue levels in commercial relevant
344 feeds was limited. It should be noted though, that the present study only assessed the parent
345 compounds of mycotoxins in limited feeding trials with ambient feed contaminations. More
346 detailed studies on the toxico-kinetics of dietary mycotoxins and their metabolites in the
347 main EU farmed fish species are needed to provide an appropriate risk assessment of food
348 safety from mycotoxin contaminated aquafeeds. More importantly, assessment on the
349 adverse effects of dietary mycotoxins on fish health and welfare is needed for the main EU
350 farmed fish species in order to establish acceptable feed mycotoxin levels for farmed fish
351 (Manning et al., 2005; Bernhoft et al., 2013).

352

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376 **References**

- 377 Aberg, A.T., Solyakov, A., Bondesson, U., 2013. Development and in-house validation
378 of an LC-MS/MS method for the quantification of the mycotoxins deoxynivalenol,
379 zearalenone, T-2 and HT-2 toxin, ochratoxin A and fumonisin B1 and B2 in
380 vegetable animal feed. *Food Addit. Contam. Part A-Chem.* 30, 541-549.
- 381 Accensi, F., Abarca, M.L., Cabanes, F.J., 2004. Occurrence of *Aspergillus* species in
382 mixed feeds and component raw materials and their ability to produce ochratoxin A.
383 *Food Microbiol.* 21, 623-627.
- 384 Afsah-Hejri, L., Jinap, S., Hajeb, P., Radu, S., Shakibazadeh, S., 2013. A Review on
385 Mycotoxins in Food and Feed: Malaysia Case Study. *Compr. Rev. Food. Sci. Food*
386 *Saf.* 12, 629-651.
- 387 Arukwe, A., Grotmol, T., Haugen, T.B., Knudsen, F.R., Goksoyr, A., 1999. Fish model
388 for assessing the in vivo estrogenic potency of the mycotoxin zearalenone and its
389 metabolites. *Sci. Total Environ.* 236, 153-161.
- 390 Baker, S.E., 2006. *Aspergillus niger* genomics: Past, present and into the future. *Med.*
391 *Mycol.* 44, S17-S21.
- 392 Bayman, P., Baker, J.L., 2006. Ochratoxins: A global perspective. *Mycopathologia* 162,
393 215-223.
- 394 Beltrán, E., Ibañez, M., Sancho, J.V., Hernandez, F., 2009. Determination of
395 mycotoxins in different food commodities by ultra-high-pressure liquid
396 chromatography coupled to triple quadrupole mass spectrometry *Rapid Commun.*
397 *Mass Spectrom.* 23, 1801–1809
- 398
399 Beltrán, E., Ibanez, M., Portoles, T., Ripolles, C., Sancho, J.V., Yusa, V., Marin, S.,
400 Hernandez, F., 2013. Development of sensitive and rapid analytical methodology for

401 food analysis of 18 mycotoxins included in a total diet study. *Anal. Chim. Acta* 783,
402 39-48.

403 Benedito-Palos, L., Navarro, J.C., Sitja-Bobadilla, A., Bell, J.G., Kaushik, S., Perez-
404 Sanchez, J., 2008. High levels of vegetable oils in plant protein-rich diets fed to
405 gilthead sea bream (*Sparus aurata* L.): growth performance, muscle fatty acid profiles
406 and histological alterations of target tissues. *Br. J. Nutr.* 100, 992-1003.

407 Bernhoft, A., Sundstøl Eriksen, G., Sundheim, L., Berntssen, M.H.G., Brantsæter, A.L.,
408 Brodal, G., Kruse Fæste, C., Skow Hofgaard, I., Rafoss, T., Sivertsen, T., Tronsmo,
409 A.M., 2013. Risk assessment of mycotoxins in cereal grain in Norway. Norwegian
410 Scientific Committee for Food Safety (VKM) 109-211,
411 <http://www.vkm.no/dav/eee04d10c04.pdf>.

412 Bertuzzi, T., Gualla, A., Morlacchini, M., Pietri, A., 2013. Direct and indirect
413 contamination with ochratoxin A of ripened pork products. *Food Control* 34, 79-83.

414 Binder, E.M., 2007a. Managing the risk of mycotoxins in modern feed production.
415 *Anim. Feed Sci. Technol.* 133, 149-166.

416 Binder, E.M., Tan, L.M., Chin, L.J., Handl, J., Richard, J., 2007b. Worldwide
417 occurrence of mycotoxins in commodities, feeds and feed ingredients. *Anim. Feed*
418 *Sci. Technol.* 137, 265-282.

419 Bryden, W.L., 2012. Mycotoxin contamination of the feed supply chain: Implications
420 for animal productivity and feed security. *Anim. Feed Sci. Technol.* 173, 134-158.

421 Burr, G.S., Wolters, W.R., Barrows, F.T., Hardy, R.W., 2012. Replacing fishmeal with
422 blends of alternative proteins on growth performance of rainbow trout
423 (*Oncorhynchus mykiss*), and early or late stage juvenile Atlantic salmon (*Salmo*
424 *salar*). *Aquaculture* 334, 110-116.

425 Caruso, D., Talamond, P., Moreau, Y., 2013. Mycotoxins and fish farming: A risk left
426 behind? *Cah. Agric.* 22, 165-173.

427 Cheli, F., Battaglia, D., Gallo, R., Dell'Orto, V., 2014. EU legislation on cereal safety:
428 An update with a focus on mycotoxins. *Food Control* 37, 315-325.

429 da Rocha, M.E.B., Freire, F.D.O., Maia, F.B.F., Guedes, M.I.F., Rondina, D., 2014.
430 Mycotoxins and their effects on human and animal health. *Food Control* 36, 159-165.

431 Davies, S.J., Gouveia, A., Laporte, J., Woodgate, S.L., Nates, S., 2009. Nutrient
432 digestibility profile of premium (category III grade) animal protein by-products for
433 temperate marine fish species (European sea bass, gilthead sea bream and turbot).
434 *Aquac. Res.* 40, 1759-1769.

435 EC, 2001. Regulation (EC) no 999/2001 of the european parliament and of the council
436 of 22 May 2001 laying down rules for the prevention, control and eradication of
437 certain transmissible spongiform encephalopathies. *Official Journal of the European*
438 *Union L 147*, 1-38.

439 EC, 2006. Commission Recommendation No 2006/576 of 17 August 2006 on the
440 presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and
441 fumonisins in products intended for animal feeding. *Official Journal of the European*
442 *Union L 229*, 7-9.

443 EC, 2009. Regulation (EC) No 1069/2009 of the european parliament and of the council
444 of of 21 October 2009 laying down health rules as regards animal by-products and
445 derived products not intended for human consumption and repealing Regulation (EC)
446 No 1774/2002 (Animal by-products Regulation). *Official Journal of the European*
447 *Union L300*, 1-33.

448 EC, 2013a. Commission regulation (EU) No 56/2013 of 16 January 2013 amending
449 Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and

450 of the Council laying down rules for the prevention, control and eradication of
451 certain transmissible spongiform encephalopathies. Official Journal of the European
452 Union 21, 3-16.

453 EC, 2013b. Commission Recommendation No 2013/165/EU of 27 March 2013 on the
454 presence of T-2 and HT-2 toxin in cereals and cereal products. Official Journal of the
455 European Union L 91, 12–15.

456 EFSA, 2004a. Opinion of the Scientific Panel on Contaminants in Food Chain on a
457 request from the Commission related to ochratoxin A (OTA) as undesirable
458 substance in animal feed Request No EFSA-Q-2003-039 Adopted on 22 September
459 2004. The EFSA Journal 101, , 1-36.

460 EFSA, 2004b. Opinion of the Scientific Panel on Contaminants in the Food Chain on a
461 request from the Commission related to Zearalenone as undesirable substance in
462 animal feed (Question N° EFSA-Q-2003-037 Adopted on 28 July 2004. The EFSA
463 journal 89, 1-35.

464 EFSA, 2005. Opinion of the Scientific Panel on Contaminants in Food Chain on a
465 request from the Commission related to fumonisins as undesirable substances in
466 animal feed Request No. EFSA-Q-2003-040. The EFSA Journal 235.

467 EFSA, 2007. Opinion of the Scientific Panel on Contaminants in the Food Chain on a
468 request from the Commission related to Deoxynivalenol (DON) as undesirable
469 substance in animal feed (Question N° EFSA-Q-2003-036) Adopted on 2 June 2004
470 adapted 2007. The EFSA Journal (2004) 73, 1-42, 1-42.

471 EFSA, 2011. Scientific Opinion on the risks for animal and public health related to the
472 presence of T-2 and HT-2 toxin in food and feed¹. The EFSA journal 9, 2481.

473 EU, 2002. Directive 2002/32/EC of the European parliament and of the council of 7
474 May 2002 on undesirable substances in animal feed. Off. J. Eur. Commun L140, 10-
475 22.

476 Frisvad, J.C., Smedsgaard, J., Samson, R.A., Larsen, T.O., Thrane, U., 2007. Fumonisin
477 B(2) production by *Aspergillus niger*. J. Agric. Food Chem. 55, 9727-9732.

478 Hooft, J.M., Elmor, H.I., Encarnacao, P., Bureau, D.P., 2011. Rainbow trout
479 (*Oncorhynchus mykiss*) is extremely sensitive to the feed-borne *Fusarium* mycotoxin
480 deoxynivalenol (DON). Aquaculture 311, 224-232.

481 Larsen, T.O., Svendsen, A., Smedsgaard, J., 2001. Biochemical characterization of
482 ochratoxin A-producing strains of the genus *Penicillium*. Appl. Environ. Microbiol.
483 67, 3630-3635.

484 Leeman, W.R., Van den Berg, K.J., Houben, G.F., 2007. Transfer of chemicals from
485 feed to animal products: The use of transfer factors in risk assessment. Food Addit.
486 Contam. 24, 1-13.

487 Lund, F., Frisvad, J.C., 2003. *Penicillium verrucosum* in wheat and barley indicates
488 presence of ochratoxin A. J. Appl. Microbiol. 95, 1117-1123.

489 Magan, N., Aldred, D., Mylona, K., Lambert, R.J.W., 2010. Limiting mycotoxins in
490 stored wheat. Food Addit. Contam. Part A-Chem. 27, 644-650.

491 Malachová, A., Sulyok, M., Beltrán, E., Berthiller, F., Krska, R., 2014. Optimization
492 and validation of a quantitative liquid chromatography–tandem mass spectrometric
493 method covering 295 bacterial and fungal metabolites including all regulated
494 mycotoxins in four model food matrices. J. Chromatogr. A.
495 DOI:10.1016/j.chroma.2014.08.037

496 Malagutti, L., Zannotti, M., Scampini, A., Sciaraffia, F., 2005. Effects of Ochratoxin A
497 on heavy pig production. Anim. Res. 54, 179-184.

498 Manning, B.B., Li, M.H., Robinson, E.H., Gaunt, P.S., Camus, A.C., Rottinghaus, G.E.,
499 2003. Response of channel catfish to diets containing T-2 toxin. *J. Aquat. Anim.*
500 *Health* 15, 229-238.

501 Manning, B.B., Li, M.H., Robinson, E.H., 2005. Feedborne mycotoxins in aquaculture
502 feeds: impact and management of aflatoxin, fumonisin, and moniliformin.

503 Mizáková, A., Pipová, M., Turek, P., 2002. The occurrence of moulds in fermented raw
504 meat products. *Czech Journal of Food Sciences* 20 89-94.

505 Mogensen, J.M., Nielsen, K.F., Samson, R.A., Frisvad, J.C., Thrane, U., 2009. Effect of
506 temperature and water activity on the production of fumonisins by *Aspergillus niger*
507 and different *Fusarium* species. *BMC Microbiol.* 9.

508 Monbaliu, S., Van Peteghem, C., De Saeger, S., 2012. Detection and determination of
509 natural toxins (mycotoxins and plant toxins) in feed. in: FinkGremmels, J. (Ed.).
510 *Animal Feed Contamination: Effects on Livestock and Food Safety.* Woodhead Publ
511 Ltd, Cambridge, pp. 286-325.

512 Monbaliu, S., Van Poucke, C., Detavernier, C., Dumoulin, F., Van De Velde, M.,
513 Schoeters, E., Van Dyck, S., Averkieva, O., Van Peteghem, C., De Saeger, S., 2010.
514 Occurrence of Mycotoxins in Feed as Analyzed by a Multi-Mycotoxin LC-MS/MS
515 Method. *J. Agric. Food Chem.* 58, 66-71.

516 Nacher-Mestre, J., Ibanez, M., Serrano, R., Perez-Sanchez, J., Hernandez, F., 2013.
517 Qualitative Screening of Undesirable Compounds from Feeds to Fish by Liquid
518 Chromatography Coupled to Mass Spectrometry. *J. Agric. Food Chem.* 61, 2077-
519 2087.

520 Njobeh, P.B., Dutton, M.F., Aberg, A.T., Haggblom, P., 2012. Estimation of Multi-
521 Mycotoxin Contamination in South African Compound Feeds. *Toxins* 4, 836-848.

522 Noonim, P., Mahakarnchanakul, W., Nielsen, K.F., Frisvad, J.C., Samson, R.A., 2009.
523 Fumonisin B2 production by *Aspergillus niger* in Thai coffee beans. *Food Addit.*
524 *Contam. Part A-Chem.* 26, 94-100.

525 Ostry, V., Malir, F., Ruprich, J., 2013. Producers and Important Dietary Sources of
526 Ochratoxin A and Citrinin. *Toxins* 5, 1574-1586.

527 Pietsch, C., Kersten, S., Burkhardt-Holm, P., Valenta, H., Danicke, S., 2013.
528 Occurrence of Deoxynivalenol and Zearalenone in Commercial Fish Feed: An Initial
529 Study. *Toxins* 5, 184-192.

530 Pitt, J.I., Hocking, A.D., 2009. *Fungi and Food Spoilage*, Third Edition. Springer, New
531 York.

532 Pitt, J.I., Taniwaki, M.H., Cole, M.B., 2013. Mycotoxin production in major crops as
533 influenced by growing, harvesting, storage and processing, with emphasis on the
534 achievement of Food Safety Objectives. *Food Control* 32, 205-215.

535 Poston, H., Coffin, J., Combs, G., 1982. Biological effects of dietary T-2 toxin on
536 rainbow-trout, *Salmo-gairdneri*. *Aquat. Toxicol.* 2, 79-88.

537 Rodrigues, I., Naehrer, K., 2012. A Three-Year Survey on the Worldwide Occurrence
538 of Mycotoxins in Feedstuffs and Feed. *Toxins* 4, 663-675.

539 Schatzmayr, G., Streit, E., 2013. Global occurrence of mycotoxins in the food and feed
540 chain: facts and figures. *World Mycotoxin J.* 6, 213-222.

541 Schmidt-Heydt, M., Graf, E., Stoll, D., Geisen, R., 2012. The biosynthesis of ochratoxin
542 A by *Penicillium* as one mechanism for adaptation to NaCl rich foods. *Food*
543 *Microbiol.* 29, 233-241.

544 Scudamore, K.A., Livesey, C.T., 1998. Occurrence and significance of mycotoxins in
545 forage crops and silage: a review. *J. Sci. Food Agric.* 77, 1-17.

546 Siegel, D., Babuscio, T., 2011. Mycotoxin management in the European cereal trading
547 sector. *Food Control* 22, 1145-1153.

548 Soares, C., Calado, T., Venancio, A., 2013. Mycotoxin production by *Aspergillus niger*
549 aggregate strains isolated from harvested maize in three Portuguese regions. *Revista*
550 *Iberoamericana De Micologia* 30, 9-13.

551 Sonjak, S., Licen, M., Frisvad, J.C., Gunde-Cimerman, N., 2011. Salting of dry-cured
552 meat - A potential cause of contamination with the ochratoxin A-producing species
553 *Penicillium nordicum*. *Food Microbiol.* 28, 1111-1116.

554 Sorensen, L.M., Mogensen, J., Nielsen, K.F., 2010. Simultaneous determination of
555 ochratoxin A, mycophenolic acid and fumonisin B-2 in meat products. *Anal.*
556 *Bioanal. Chem.* 398, 1535-1542.

557 Streit, E., Schatzmayr, G., Tassis, P., Tzika, E., Marin, D., Taranu, I., Tabuc, C.,
558 Nicolau, A., Aprodu, I., Puel, O., Oswald, I.P., 2012. Current Situation of Mycotoxin
559 Contamination and Co-occurrence in Animal Feed-Focus on Europe. *Toxins* 4, 788-
560 809.

561 Streit, E., Naehrer, K., Rodrigues, I., Schatzmayr, G., 2013. Mycotoxin occurrence in
562 feed and feed raw materials worldwide: long-term analysis with special focus on
563 Europe and Asia. *J. Sci. Food Agric.* 93, 2892-2899.

564 Tacon, A.G.J., Metian, M., 2008. Global overview on the use of fish meal and fish oil in
565 industrially compounded aquafeeds: Trends and future prospects. *Aquaculture* 285,
566 146-158.

567 Toldra, F., Aristoy, M.C., Mora, L., Reig, M., 2012. Innovations in value-addition of
568 edible meat by-products. *Meat Sci.* 92, 290-296.

569 Torrissen, O., Olsen, R.E., Toresen, R., Hemre, G.I., Tacon, A.G.J., Asche, F., Hardy,
570 R.W., Lall, S., 2011. Atlantic Salmon (*Salmo salar*): The "Super-Chicken" of the
571 Sea? Rev. Fish. Sci. 19, 257-278.

572 Torstensen, B.E., Espe, M., Sanden, M., Stubhaug, I., Waagbo, R., Hemre, G.I.,
573 Fontanillas, R., Nordgarden, U., Hevroy, E.M., Olsvik, P., Berntssen, M.H.G., 2008.
574 Novel production of Atlantic salmon (*Salmo salar*) protein based on combined
575 replacement of fish meal and fish oil with plant meal and vegetable oil blends.
576 Aquaculture 285, 193-200.

577 Varga, J., Kocsube, S., Suri, K., Szigeti, G., Szekeres, A., Varga, M., Toth, B., Bartok,
578 T., 2010. Fumonisin contamination and fumonisin producing black Aspergilli in
579 dried vine fruits of different origin. Int. J. Food Microbiol. 143, 143-149.

580 Wozny, M., Brzuzan, P., Luczynski, M.K., Gora, M., Bidzinska, J., Jurkiewicz, P.,
581 2008. Effects of cyclopenta[c]phenanthrene and its derivatives on zona radiata
582 protein, ER alpha, and CYP1A mRNA expression in liver of rainbow trout
583 (*Oncorhynchus mykiss* Walbaurn). Chem.-Biol. Interact. 174, 60-68.

584 Wozny, M., Obremski, K., Jakimiuk, E., Gusiatin, M., Brzuzan, P., 2013. Zearalenone
585 contamination in rainbow trout farms in north-eastern Poland. Aquaculture 416, 209-
586 211.

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Table 1. Levels of mycotoxins ($\mu\text{g kg}^{-1}\text{ww}$, minimum-maximum (number of positive samples)) in commercially available plant feed ingredients used in aquafeeds (n=number of different samples). - = not detectable at given matrix limit in table 2.

	Sunflower meal (n=1)	Rapeseed meal (n=1)	wheat (n=3)	wheat gluten (n=4)	corn gluten (n=3)	Pea protein (n=3)	soy protein concentrate (n=4)
AFG2	-	-	-	-	-	-	-
AFG1	-	-	-	-	-	-	-
AFB2	-	-	-	-	-	-	-
AFB1	-	-	-	-	-	-	-
NIV	-	-	-	-	-	-	-
Fus X	-	-	-	-	-	-	-
DON	-	-	53-371 (3)	17-504 (4)	139-814 (3)	-	-
3-AcDON	-	-	-	-	-	-	-
15-AcDON	-	-	-	-	53-452	-	-
NEO	-	-	-	-	-	-	-
DIA	-	-	-	-	-	-	-
HT-2	-	-	4-8.1 (2)	4 (2)	67 (1)	-	-
T-2	-	-	4 (1)	4 (2)	2.8 (1)	-	-
ZEN	-	-	-	14-17 (2)	8-13 (3)	-	-
OTA	0.4	0.4	0.4 (1)	2.0-5.2 (4)	0.4 (3)	1.8 (1)	-
FB1	-	-	-	0.4-8.2 (2)	0.4-2319 (3)	-	0.4 (2)
FB2	-	-	-	2.9 (1)	2.9-1943 (3)	-	0.5 (1)
FB3	-	-	-	2.1 (1)	7.8-638 (3)	-	-
Sum FB1+FB2+FB3	-	-	-	13.2	11.1-4901	-	-

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592 **Table 2.** Levels of mycotoxins ($\mu\text{g kg}^{-1}\text{ww}$) of two gilthead sea bream diets (GSB-D) with low or high inclusion levels of plant material (GSB-
593 D1 and GSB-D2, respectively), and three production replicates for Atlantic salmon diets with high plant ingredient inclusions levels (AS-D1-3). -
594 = not detectable at given matrix limit in table 2. None of the dietary mycotoxins were detected in the fillets of sea bream or Atlantic salmon fed
595 for respectively 8 or 7 months on these diets.

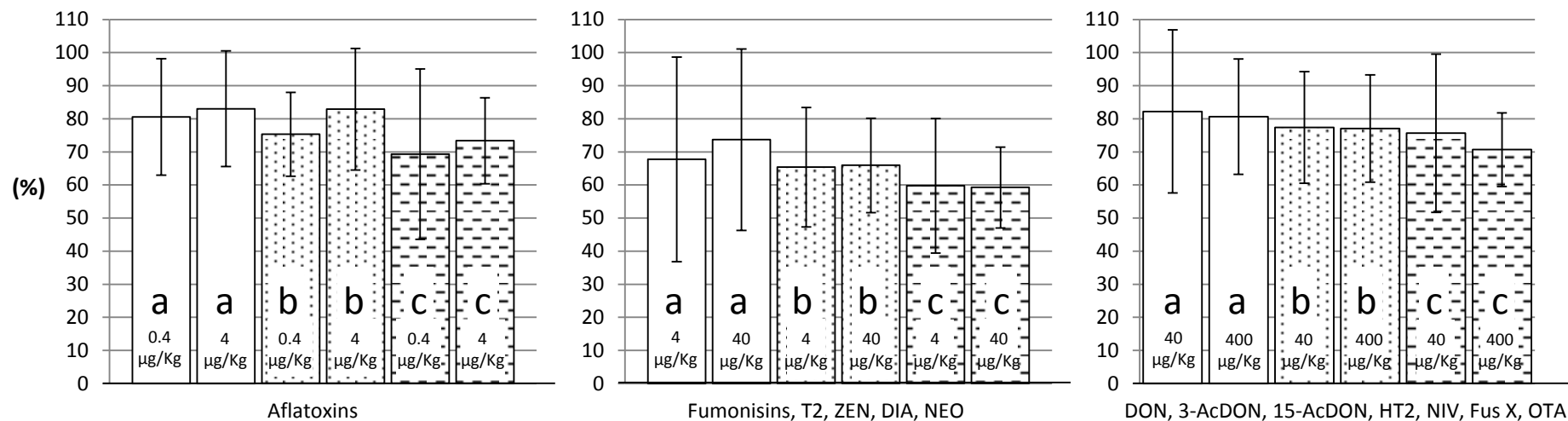
Diets	GSB-D1	GSB-D2	AS-D1	AS-D2	AS-D3
AFG2	-	-	-	-	-
AFG1	-	-	-	-	-
AFB2	-	-	-	-	-
AFB1	-	-	-	detected	detected
NIV	-	-	-	-	-
Fus X	-	-	-	-	-
NEO	-	-	-	-	-
DON	79,2	53,5	22,4	19,4	23,1
3-AcDON	-	-	-	-	-
15-AcDON	8,1	13,6	detected	detected	detected
DIA	-	-	-	-	-
HT-2	-	-	detected	5	-
T-2	-	-	0,1	0,1	0,1
ZEN	-	-	-	-	-
OTA	-	-	detected	detected	detected
FB1	-	4,5	66,9	335	50,6
FB2	-	1,9	62,2	324	43,9
FB3	-	detected	18,9	95,3	18
Sum FB1+FB2+FB3	-	6,4	148	754	112

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Figure 1. General overview about the QC recoveries in every matrix (a=ingredients, n=14; b=feeds, n=4; c=fish fillets, n=4) for the different groups of mycotoxins. The error bars represent the relative standard deviation of the different groups of analytes in the different matrices (a, b and c).