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

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Abstract

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

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

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1. Introduction

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

The use of these alternative feed ingredients can introduce contaminants that were previously not associated with marine salmon and sea bream farming. One example of this are mycotoxins, which are world-wide found in cereal grains and animal feed (Binder, 2007 a,b; Beltran et al, 2013; Streit et al., 2013). Mycotoxins are produced by fungi that pre-harvest infect agricultural crops (field mycotoxins) or post-harvest agricultural commodities stored under certain temperature and humidity conditions (storage mycotoxins) (Magan et al., 2010; Bryden, 2012). Meat products can also be contaminated with mycotoxins (Mizáková et al., 2002; Sorensen et al., 2010; Ostry et al., 2013), and animal by-products could hence be a potential source for these mycotoxins in animal feeds (Caruso et al., 2013). The mycotoxin aflatoxin B1 (AFB1) is under EU feed regulation (EU, 2002), while guidance values have been set for animal feed ingredients and animal feed for several mycotoxins, including deoxynivalenol (DON), zearalenone (ZEN), ochratoxin A (OTA), and fumonisin B1 + B2 (FB1+FB2)(EC, 2006). For other mycotoxins, such as T-2 and HT-2 toxins, indicative levels for cereal products, including those intended for animal feed have been set (EC, 2013b; Cheli et al., 2014). In fact, many surveillance studies have reported mycotoxin levels on a wide range of randomly sampled feed ingredients and finished feeds from terrestrial animals (Binder, 2007 a,b; Rodrigues and Naehrer, 2012; Streit et al., 2012; Streit et al., 2013), but only few studies recent studies are done in fish feeds or farmed fish (Pietsch et al., 2013; Wozny et al., 2013). Besides, most fish studies on mycotoxins are focused on the hazards for fish health in experimental trials with fortified feeds (Poston et al., 1982; Arukwe et al., 1999; Manning et al., 2003; EFSA, 2005; Manning et al., 2005; Wozny et al., 2008; EFSA, 2011; Hooft et al., 2011; Caruso et al., 2013) with little information on the carry-over to the edible parts of the fish.

Multi occurrence of mycotoxins requires, however, the need for the application of multi-mycotoxin methods in order to get a more accurate picture of the extent of the wide range of mycotoxin contamination (Beltran et al., 2009, 2013; Monbaliu et al., 2010; Streit et al., 2012; Aberg et al., 2013). Earlier studies established feasible analytical approaches for mycotoxins in feed ingredients, aquafeeds and fish fillets (Malachová et al., 2014; Beltran et al., 2013; Nacher-Mestre et al., 2013). Based on this previous experience, the present work aims to quantify a wide range of mycotoxins in commercially available plant and PAP feed ingredients, fish feeds based on these ingredients, and their transfer to the edible part of farmed Atlantic salmon and gilthead sea bream, two main species of the European aquaculture. In addition to the 8 mycotoxin under EU regulation/guidance in feed and feed ingredients (AFB1, DON, ZEN, OTA, FB1+FB2, T-2 and HT-2), 10 additional mycotoxins of potential relevance for food safety are included (AFB2, AFG1, AFG2, FB3, nivalenol (NIV), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), diacetoxyscirpenol (DIA), fusarenon-X (Fus X) and neosolaniol (NEO)) in the study.

2. Material and methods

2.1. Feed ingredients

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

2.2. Experimental diets and feeding trials

Fish feeds for feeding trials were based on plant feed ingredients, and not PAPs, as only noticeable mycotoxin levels were found on the former feedstuffs (see results section). The feeds were produced by Biomar under commercial aquafeed production techniques based on high-temperature extrusion processes, which potentially could affect mycotoxin residue levels. For gilthead sea bream, two diets were formulated with the same feed ingredients varying the replacement of fish meal and fish oil by plant ingredients. Salmon feeds were production triplicates of high plant ingredient diets based on the same feed ingredients (Table 1, sup. data).

Sea bream trial. Juvenile gilthead sea bream of Atlantic origin were fed with the respective diet (triplicate tanks of 2500 L in groups of 150 fish each) for 8 months (May-December) in the indoor experimental facilities of the Institute of Aquaculture of Torre la Sal (CSIC, Spain) under natural light and temperature conditions at our latitude (40º5´N; 0º10´E). Fish grew from an initial body weight of 15 g until 296-320 g with a feed:gain ratio (feed/weight gain) of 1-1.05 regardless of diet composition. Over the course of the trial, fish were fed daily (5-6 days per week) at visual satiety. At harvest (week 31), 6 fish per dietary treatment were killed by a blood to the head and deboned fillets were stored at -80 ºC until analyses.

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

2.3. Analytical procedure

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

3. Results and Discussion

The multi mycotoxin LC-ESI-MS/MS method was applied to the analysis of 18 mycotoxins in plant and animal ingredients used in the elaboration of fish feed, in different experimental feeds and in cultured fish tissues from marine aquaculture trials. Results of the QC recoveries included in each batch were in the range between 60 and 110% with few exceptions for fish fillet matrices (Table 3, supplementary material). Figure 1 shows a general overview for the QC recoveries in every matrix (ingredients, feeds and fish) for the different groups of mycotoxins (more details related to recovery values in Table 3, supplementary material). Regarding matrix effects, fumonisins, DON, OTA and ZEN were the compounds which showed higher matrix suppression in all matrices studied. LOQs at concentrations around the level of µg kg^{-1} were obtained for almost all studied mycotoxins (Table 4, supplementary material). For two mycotoxins no proper quantification could be obtained for some matrices (NIV in rapeseed, corn, pea, poultry feather and blood meal and ZEN in poultry feather and blood meal and pork meal) due to the presence of coeluted matrix interference peaks. The LOQs for the different ingredients, feeds and fish muscle from the feeding experiments were in all cases below the maximum permitted levels (EU, 2002; EC, 2006, 2013b).

3.1. Feed ingredients of plant origin

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

Plant feed ingredients for aquafeeds are sourced from the global market and in the present study ingredients were obtained from Asia, South-America and central and Northern Europe. The current study included only a limited number of possible plant feed ingredients used for aquafeeds, not providing a basis for global mycotoxin contamination assessment. Other studies on plant feed ingredients used for terrestrial animal feeds, however, have performed a far more extensive global surveillance showing regional and plant specific differences in mycotoxin contamination (Binder et al., 2007b; Monbaliu et al., 2012; Njobeh et al., 2012; Rodrigues and Naehrer, 2012; Afsah-Hejri et al., 2013; Schatzmayr and Streit, 2013; Streit et al., 2013).

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

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

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

3.2. Feed ingredients of animal origin

As expected, only the typical storage mycotoxins, OTA (at concentrations below 0.4 µg kg^{-1}) but also FB1 (at concentrations between 0.4-2.6 µg kg^{-1}), were detected in poultry feather and bone and meat meal (fumonisin) and pork blood (OTA), respectively (Table 5, supplementary material). The levels were, however, around detection limit and are by far under the EU guidelines for plant products intended for animal feeds (60 mg kg^{-1} for FB1+FB2 in maize and 250 µg kg^{-1} for OTA in cereals (EC, 2006)). Fumonisins are mainly produced by a small number of *Fusarium* species, which have specific crops (corn) as habitat (Pitt and Hocking, 2009). However, other fumonisin producing fungi such as *Aspergillus niger* (Mogensen et al., 2009) has been isolated from warm air-dried meat products (Mizáková et al., 2002; Sorensen et al., 2010). The most common fumonisin produced by *Aspergillus niger* is FB2 at high amounts of carbohydrate or NaCl (Frisvad et al., 2007), although additional FB4 can be produced in agar cultures (Noonim et al., 2009) and other fumonisins forms (FB1-4) are found on *A. niger* contaminated dried raisins (Varga et al., 2010). In the present study, FB1 was the only fumonisin form detected on PAP material albeit at low levels. The absence of FB2-3 might be due to the LOQs, which were higher than for FB1 in PAP material such as poultry meal. The *Aspergillus niger* strains are also known to produce OTA (Accensi et al., 2004), which could be a source for the detected OTA in one of the PAP samples. The fungi *Penicillium nordicum* is the most known OTA producer (Larsen et al., 2001; Lund and Frisvad, 2003) and grows well at low temperatures on meat products but mostly only at increased salinity (Schmidt-Heydt et al., 2012). Storage OTA contamination by *P. nordicum* is, therefore, often limited to salted meat food products such as cured ham and sausage (Sonjak et al., 2011; Schmidt-Heydt et al., 2012). Products of animal origin such as pork and poultry raw meat or blood products can be also indirectly contaminated by OTA when monogastric animals are fed with contaminated feed stuffs (EFSA, 2004a) as dietary OTA can be transferred from the feed to animal meat (Malagutti et al., 2005). From the present study, however, the risk of OTA or FB1+B2+B3 contamination of EU produced PAP products intended for aquafeeds seems low. Similarly, from surveillance studies on foodstuffs of both plant and animal origin it was concluded that plant products rather than cured animal products could be contaminated with OTA (Bertuzzi et al., 2013; Ostry et al., 2013)

3.3. Feed and fish muscle

The present study assess the transfer of mycotoxins throughout the sea bream and Atlantic salmon food production chain by assessing mycotoxin levels in feed ingredients and follow their transfer to commercially produced aquafeeds and eventually carry-over to the edible parts of the fish fed on these feeds. Commercially produced aquafeeds were made based on the same analysed plant feed ingredients given in table 1. Table 2 gives the mycotoxin levels of sea bream feed with either a low or high overall plant protein content as well as three Atlantic salmon feed production repeats with a similar high-plant feed composition based on the same batch of feed ingredients. For the sea bream feeds, the low plant-protein feeds had an unexpected higher DON level than high-plant protein based feeds. One of the main sources for DON in sea bream diets was contaminated wheat (371 µg kg^{-1}), which inclusion levels in low plant feed was slightly higher than high plant feed (11 versus 7 %, respectively), thus explaining the slightly higher levels in low-plant diets. The wheat gluten used in the sea bream diets had only minor DON levels (17 µg kg^{-1}). For the sea bream feeds, the main plant-protein increase in high plant protein diets came from corn and soya (from 31% to 50 % : low plant feed with 15% and 16% and high plant feed with 25% and 25% for corn and soya, respectively). The soy protein concentrate (SPC) feed ingredient had only detectable levels of FB1 and FB2, while corn was the main source for fumonisin (139 µg kg^{-1}sum FB1+2+3) and 15 Ac-DON (53 µg kg^{-1}), causing an increase in these mycotoxins in high plant-protein feeds. The differences in mycotoxin contamination of traditional marine feeds and high plant-protein substitution feeds in the present study, exemplifies that mycotoxin levels in plant-protein based feeds are more dependent on the individual contamination level of each plant-protein ingredient rather than the overall higher inclusion level of plant protein. In addition to the substitution of fish-meal with plant-proteins, an extra sea bream feed was produced in which fish oil was substituted with plant oils. This substitution had no effect on feed mycotoxin level supporting the notion that the plant proteins and not the plant oils are the main source for mycotoxin contamination.

For the Atlantic salmon high plant-protein feed production repeats, mycotoxin levels were as expected from the contamination level of the feed ingredients and with similar levels among the repeats with the exception of fumonisins. Higher feed fumonisin levels were found than could be expected from the low inclusion level (4%) of the sole fumonisin feed ingredient source (corn, 403 µg kg^{-1} sum FB1+2+3), and with a large variation (112-754 µg kg^{-1} sum FB1+2+3) among the production repeats. The large variation in fumonisin levels suggest the present of storage fungi that can grow heterogeneously within and among feed batches. The main source for fumonisins in corn are *Fusarium* species which normally grow very little under storage conditions and storage is not expected to increase *furasium* derived fumonisin contamination (Pitt et al., 2013). *Aspergilles niger* fungi species can also produce fumonisins (Baker, 2006) but they are also the source for the typical storage mycotoxin OTA, which was were only present at detectable levels in the salmon feeds as could be excepted from the inclusion of OTA contaminated pea proteins (1.8 µg kg^{-1} at inclusion level of 13%). Surveillance of finished feed for terrestrial animals in Europe and the Mediterranean area gave average fumonisin levels of 638 µg kg^{-1} in 3 out of 10 analysed samples (Binder, 2007b). Slovenian poultry feed had fumonisin levels ranging from 36-1160 µg kg^{-1} (Streit et al., 2012). Surveillance of feed ingredients and finished feeds in Europe and the Mediterranean showed maximum OTA level in feed ingredients to be 33 µg kg^{-1} while in finished feeds the mean levels were 305 µg kg^{-1} with maximum of 530 µg kg^{-1}, thus suggesting OTA contamination during storage of finished feeds. Studies on rainbow trout feeds in Poland showed ZEN contamination up to 82 µg kg^{-1}; in the present study however ZEN was not detected in any of the feeds.

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

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Table 1. Levels of mycotoxins (µg kg^{-1}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	-	-

Table 2. Levels of mycotoxins (µg kg^{-1}ww) 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 high plant ingredient inclusions levels (AS-D1-3). - = 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 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

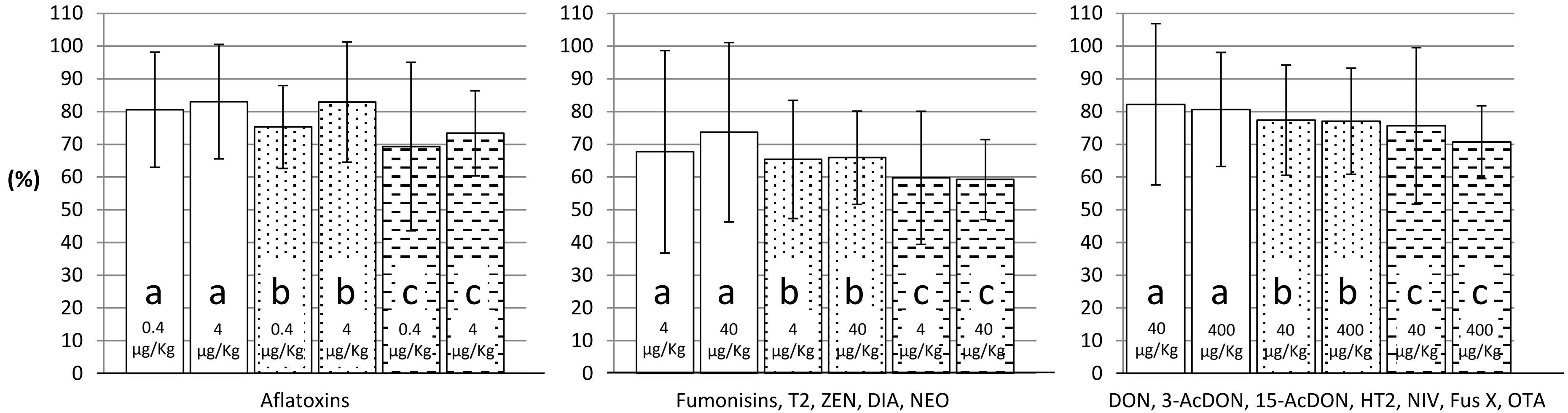

Figure 1. General overview about the QC recoveries in every matrix (a=ingredients, n=14; b=feeds, n=4; c=fish fillets, n=4) for the different groups of mycotoxins. The error bars represent the relative standard deviation of the different groups of analytes in the different matrices (a, b and c).