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

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HIGHLIGHTS

- Occurrence of mycotoxins in feeds made from plants and animal by-products.
- Carry-over from feeds to fillets of Atlantic salmon and gilthead sea bream.
- None observed mycotoxins carry-over from feed to edible parts of fish.
- Discussion of the use of plant and animal ingredients in fish feeding.
- Satisfactory limits of quantification below LMR established by EU regulations.

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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 $\mu g\,kg^{-1}$, followed by fumonisins in corn products (range 11.1–4901 $\mu g\,kg^{-1}$ 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.

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

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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; Binder et al., 2007; Beltrán 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; Binder et al., 2007; Rodrigues and Naehrer, 2012; Streit et al., 2012, 2013), but only few 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, 2005; EFSA, 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 (Beltrán 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 (Beltrán et al., 2013; Nácher-Mestre et al., 2013; Malachová et al., 2014). 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 Section 3). 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).

2.2.1. 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 (40°5′N; 0°10′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 d 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 °C until analyses.

2.2.2. Salmon trial

Post-smolts were randomly distributed among 6 sea cages (5 m × 5 m × 5 m; 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 °C 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 analysed according to the methodology of Beltrán et al. (2013), adapted to the aquaculture matrices (Nácher-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