DETERMINATION OF OCHRATOXIN A IN FARMED FISH BY ENZYMATIC DIGESTION (ED) COUPLED TO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH A FLUORESCENCE DETECTOR (HPLC-FLD)

Valentina Meucci, Giacomo Luci, Andrea Armani, Lisa Guardone, Luigi Intorre

Università di Pisa, Dipartimento di Scienze Veterinarie

Several studies have demonstrated that fish feeds contain significant concentrations of chemical contaminants, many of which can bioaccumulate and bioconcentrate in fish tissues [1]. The serious concern regarding the use of fish meal and fish oil in the aquaculture industry has led to extensive search of alternative raw materials for aquafeeds. The most obvious alternatives are oils and proteins of plant origin. The use of these alternative feed ingredients can introduce contaminants that were previously not associated with fish farming such as mycotoxins [2]. Ochratoxin A (OTA) is a mycotoxin produced as a secondary metabolite by various *Aspergillus* and *Penicillium* species with nephrotoxic, carcinogenic, immunotoxic and teratogenic potential [3]. OTA has been found in several food commodities, including cereals and can also be present in food of animal origin as a result of carryover from contaminated feed [3]. The aim of the present study was to determine OTA concentrations in muscle, kidney and liver of 10 seabream and 10 seabass of farmed origin collected on the market. Analysis will be performed by using an enzymatic digestion (ED) method coupled to high-performance liquid chromatography with a fluorescence detector (HPLC-FLD).

Fish tissues were digested for 1 hour at 37°C with a 1% pancreatin solution in a phosphate buffer and then cleaned up with ethylacetate. After being evaporated to dryness and re-dissolved, the sample was processed using HPLC-FLD. The method was validated for: specificity, recovery, trueness, selectivity, linearity, limit of detection (LOD) and limit of quantification (LOQ), repeatability and reproducibility.

Recoveries of analytical method were higher than 85% for all the matrices. Intra- and inter-day repeatability expressed as relative standard deviation were less than 9%. The LOD and LOQ for liver and muscles samples were 0.001 and 0.002 µg/kg, respectively. The LOD and LOQ for kidney samples were 0.01 and 0.02 µg/kg, respectively.

The highest concentrations of OTA were found in the kidney of the 20 fish analyzed (range <LOD-0.91 µg/kg, mean 0.32 ± 0.30 µg/kg). The concentrations found in the liver ranged between <LOD-0.74 µg/kg, (mean 0.53 ± 0.22 µg/kg). The lowest concentrations were found in muscle (<LOD-0.28 µg/kg, mean 0.12 ± 0.11 µg/kg). No differences were found between the two analysed species.

The present results are in agreement with previous studies [4] suggesting that an high OTA amount could be present in feed administered to fish sampled in this study.

[1] Jacobs et al., Investigation of selected persistent organic pollutants in farmed Atlantic salmon (*Salmo salar*), salmon aquaculture feed, and fish oil components of the feed. Environmental Science and Technology, 36:2797-805, 2002. [2] Náscher-Mestre et al. Occurrence and potential transfer of mycotoxins in gilthead sea bream and Atlantic Salmon by use of novel alternative feed ingredients. Chemosphere, 128:314-320, 2015. [3] Malir et al. Ochratoxin A: 50 Years of Research. Toxins 8:191, 2016. [4] Bernhoft et al., Tissue distribution and elimination of deoxynivalenol and ochratoxin A in dietary-exposed Atlantic salmon (*Salmo salar*), Food Additives & Contaminants: Part A, DOI: 10.1080/19440049.2017.1321149.