Mycotoxins in Aquaculture: Feed and Food

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Abstract

Mycotoxins, secondary metabolites produced by molds, are responsible for causing significant economic losses due to spoilage of agricultural products but also due to direct or indirect health impact on livestock upon ingestion of mycotoxin contaminated feedstuffs. Aquaculture farmed species are not an exception and studies reporting mycotoxin-related issues in the aquaculture industry have been increasing. However, our understanding on the prevalence and impact of mycotoxins in the aquaculture sector is still lower compared to the terrestrial livestock sector. Consequently, regulatory limits and guidance values have been defined based on the studies on terrestrial farm animals.

The aim of this review is to compile and critically assess mycotoxin occurrence and co-occurrence in aquaculture finished feeds, and understand the risk of mycotoxin carry-over in aquaculture seafood products. Furthermore, we aim with this review to raise awareness to the scientific community, the regulatory authorities and the aquaculture industry to the need for specific aquaculture mycotoxin maximum concentration levels for both aquaculture feeds and foods.

Keywords: mycotoxins occurrence; carry-over effects; fish; shrimp; aquafeeds; transfer factor

1

2   **Mycotoxin abbreviations:**

3   AFs: aflatoxins; meaning the sum of AFB_1, AFB_2, AFG_1 and AFG_2

4   AFB_1: aflatoxin B_1

5   AFB_2: aflatoxin B_2

6   AFG_1: aflatoxin G_1

7   AFG_2: aflatoxin G_2

8   DON: deoxynivalenol

9   ENNs: enniatins

10 FUM: fumonisins; meaning the sum of FB_1 and FB_2

11 FB_1: fumonisin B_1

12 FB_2: fumonisin B_2

13 OTA: ochratoxin A

14 ZEN: zearalenone

15 α-ZEL: alpha-Zearalenol

16 β-ZEL: beta-Zearalenol

17

18 **Other abbreviations:**

19 TF – Transfer factor

20 DN – Denmark

21 AT – Austria

22 NL – the Netherlands

23 DE – Germany

24 VN – Vietnam

25 ID – Indonesia

26 MM - Myanmar

27   **INTRODUCTION**

28   Mycotoxins are secondary metabolites produced by some molds (Hussein and Brasel, 2001).

29   These can be produced on agricultural commodities pre- and/or post-harvest including

30   directly in finished feeds. Mycotoxins are responsible for significant economic losses due to

31   the spoilage of agricultural products (CAST, 2003; Shane and Eaton, 1994; Vasanthi and Bhat,

32   1998). Furthermore, mycotoxins can cause diseases problems when consumed by humans

33   and livestock, causing significant problems worldwide (Zain, 2011). Despite being identified

34   as categorically undesirable for most aquaculture species, their occurrence, at least in field

35   conditions, is not completely preventable even when using good manufacturing practices

36   (FAO 1979). The awareness of mycotoxin-related issues in the aquaculture industry has been

37   increasing, accentuated by the increased inclusion levels of plant meals in aquafeeds (Tacon

38   *et al.* 2011). Traditionally, the use of minor amounts of plant feed stuffs led to an accepted

39   perception that mycotoxins were not a relevant issue in aquaculture and that the majority of

40   mycotoxin issues would stemmed only due to poor storage conditions. *Aspergillus* spp. and

41   *Penicillium* spp. can grow on feed stored in poor conditions, ultimately leading to the

42   production of aflatoxin (AF) and ochratoxin A (OTA). This would seem to be particularly the

43   case in countries where climate conditions are favourable to the growth of *Aspergillus* spp.

44   and *Penicillium* spp. fungi. However, optimal storage conditions should prevent the

45   contamination of raw materials and finished feeds from AF or OTA. However, some plant

46   commodities such as cottonseed and peanut meals commonly present detectable levels of

47   AF and/or OTA (Gonçalves *et al.* 2017), even when stored using appropriate conditions.

48   With the increased use of plant meals in aquafeeds, other mycotoxins besides AF and OTA

49   have been reported in finished feeds, as mycotoxins are reasonably stable to processing

50   conditions (Cheli *et al.* 2013). *Fusarium* mycotoxins (Type B and A, trichothecenes and

51   fumonisins) are, contrary to AF and OTA, mainly produced at pre-harvest stage. The

52   production of these mycotoxins by *Fusarium* spp. seems to be highly influenced by

53   environmental conditions, so an increase in occurrence is expected due to climate change

54   (Miraglia *et al.* 2009; Paterson and Lima, 2010; Paterson and Lima, 2011). This contamination

55   may potentially cause harm to the fish and shrimps, dependent upon mycotoxin

56   concentration and co-occurrence, consequently resulting in significant economic losses,

57   directly (e.g., mortality or decreases in performance), or indirectly (e.g. higher susceptibility

58   to diseases). However, one of the biggest barriers to quantify the impact of mycotoxin

59 contamination in the aquaculture industry is the apparent lack of clinical signs or biomarkers

60 in aquatic species for mycotoxin exposure, especially compared to terrestrial livestock. While

61 several reports describe broad and non-specific clinical signs for the most common

62 mycotoxins (see review from Anater *et al.* (2016)), these lack specificity and could be

63 attributed to a number of pathologies or challenges such as the presence of anti-nutrition

64 factors or lectins in the diet (Hart *et al.* 2010). The case of aflatoxicosis, (yellowing of the

65 body surface, (Deng *et al.* 2010) and ingestion of fumonisins (FUM; alteration of the

66 sphinganine to sphingosine ratio, (Tuan *et al.* 2003)) are two notable exceptions. Also,

67 Gonçalves *et al*. (2018b) described DON-3-sulfate as a potential biomarker of deoxynivalenol

68 (DON) exposure in rainbow trout (*Oncorhynchus mykiss*).

69 Carry-over denotes the conveyance of undesired compounds from contaminated feed into

70 food of animal origin. The potential of carry-over of several mycotoxins in terrestrial animals

71 such as poultry, swine and cows issue was highlighted by the European Food Safety

72 Authorities (EFSA) and FAO (Domenico Caruso *et al.* 2013; EFSA, 2004b FAO, 2001)).

73 However, no guidelines are available regarding carry-over in farmed fish and shrimp species.

74 Therefore, the present review aims to compare the mycotoxin occurrence and co-

75 occurrence in aquaculture finished feeds with the potential risk of mycotoxin carry-over in

76 aquaculture seafood products across main aquaculture produced species. Furthermore, we

77 aim to critically compare carry-over obtain in aquaculture species to the ones obtained for

78 livestock species. With this review, we intend to raise awareness to the scientific community,

79 the regulatory authorities and the aquaculture industry to the possible need for specific

80 aquaculture mycotoxin maximum concentration levels for both aquaculture feeds and foods.

81 Furthermore, authors aware for particular cases in aquaculture sector, where edible tissues

82 may change in different regions, therefore increasing the risk of mycotoxicosis.

83

84 **OCCURRENCE OF MYCOTOXINS IN AQUAFEEDS**

85 The high cost and limited availability of fishmeal has led the aquaculture industry to

86 gradually increase the levels of alternative protein sources as a substitute for fishmeal in

87 their feeds (Davis and Sookying, 2009). Overall, a wide range of products, e.g. animal by-

88 products, fishery by-products, insect meals, macro-algae meals or single-cell protein, have

89 been explored as alternatives to fishmeal. However, for several reasons (e.g., production

90 scalability, market availability, batch uniformity or price competitiveness) plant-based meals

91   remain the most widely used alternative protein source. When considering plant-based

92   meals for aquafeeds, it is commonly agreed that one of the negative aspects is the presence

93   of anti-nutrients (e.g. cyanogens, saponins, tannins etc.) which are detrimental to fish and

94   shrimp (Krogdahl *et al*. 2010). Conversely, the negative impact of mycotoxins is often

95   overlooked. The disbelief in the negative effects of mycotoxins on aquatic species might be

96   related to the lack of observable clinical signs in aquatic species directly related to mycotoxin

97   ingestion compared to terrestrial livestock species where the effects are more pronounced.

98   However, the awareness of mycotoxin-related issues in the aquaculture industry has grown

99   in recent years as feed manufacturers and producers have recognised the importance of

100   mycotoxins and their potential to impact production, final product quality (García-Morales *et*

101   *al.* 2013) and safety for consumers (Michelin *et al.* 2017). The evolution of the analytical

102   platforms used to detect mycotoxins and the easier access to analytical labs or simple ELISA

103   strip tests kits for *in situ* testing, has also increased the awareness of mycotoxins to feed

104   millers and farmers.

105   During the revision of the peer-reviewed literature on the occurrence of mycotoxins in

106   aquafeeds, summarized in this review, a pattern of the target mycotoxins analysed in feed

107   samples emerged. In samples analysed before 2012, the main mycotoxins analysed were AFs

108   (AFB$_1$, AFB$_2$, AFG$_1$, AFG$_2$; in most of the cases only AFB$_1$; see Table 1) and in some cases

109   zeralenone (ZEN) and OTA (Fegan and Spring 2007) (with the exception of (Martins *et al.*

110   2008) and, possibly based on previous data reported on terrestrial livestock feed samples.

111   After 2012, other mycotoxins were beginning to be reported besides AF's (Table 1). These

112   studies have either targeted the analysis of specific mycotoxins due to the inclusion of

113   certain plant meals (e.g., (Pietsch *et al.* 2013; Woźny *et al.* 2013) or explored a broad

114   mycotoxin occurrence (Gonçalves *et al.* 2018a; Gonçalves *et al.* 2018; Gonçalves *et al.* 2017;

115   Nácher-Mestre *et al.* 2015). This different pattern in the target mycotoxin analysed in feed

116   might be a reflection of increasing awareness of mycotoxins in aquaculture, but also as a

117   result of the easier access to mycotoxin analytical methods.

118

119   Aquafeed studies with samples preceding 2012

120   The oldest documented survey of mycotoxin occurrence in aquaculture finished feed was by

121   Bautista *et al.* (1994). In this study, a total of 62 samples collected in the Philippines between

122   August 1990 to February 1991 from black tiger shrimp (*Penaeus monodon*) feed, sourced

123   from feed mills and at farm level were analysis (Table 1). The authors observed that only two

124   of the 62 samples were free from AFs, 36 samples were contaminated with AFs at levels

125   between 10 and 20 µg kg^{-1}, 21 samples contained AFs at levels between 30 and 40 µg kg^{-1}

126   and two samples had AFs levels of 60 and 120 µg kg^{-1}. The second study was from Bintvihok

127   *et al*. (2003) which analysed samples collected in the eastern and southern regions of

128   Thailand (1997 to 1998) and by Altuğ and Berklevik (2001) with samples collected in Turkey

129   from 1998 to 2000 (Table 1). Bintvihok *et al*. (2003) analysed 150 samples of commercial

130   shrimp feed (formulated for *Penaeus monodon*) composed mainly of fishmeal, soybean and

131   corn. Samples were collected directly from farms in ten different provinces during the

132   summer months (March to June 1997), the rainy season (July to October 1997) and the

133   winter (November to February 1998) and analysed for AFB$_1$, AFB$_2$, AFG$_1$ and AFG$_2$. Bintvihok

134   *et al*. (2003) observed that feed was more frequently contaminated in the eastern region (43

135   contaminated out of 75 collected samples) compared to the southern region (14

136   contaminated out of 75 collected samples). Contamination also occurred more frequently

137   during rainy season (29 contaminated out of 50 collected samples) followed by winter (20

138   contaminated in 50 collected samples). AFB$_1$ was the most prevalent mycotoxin found in

139   samples, although at relatively low concentrations (< 1 µg kg^{-1}; Table 1). However, the study

140   lacked information regarding levels of inclusion of the plant ingredients as well as storage

141   time and conditions prior to analysis, which does not allow drawing further conclusions

142   regarding the origin of the AF contamination (i.e., from raw materials or contamination

143   during storage). Soybean and corn are not typically contaminated with AFs, at least in the

144   field, as these plant commodities are more likely contaminated with DON, FUM and ZEN

145   (Gonçalves *et al.* 2018a). Therefore, AF contamination in finished feeds could reflect

146   inadequate storage conditions of raw materials or feeds. Reporting inclusion levels of plant

147   ingredients would be very useful. Importantly, Altuğ and Berklevik (2001) analysed 170 fish

148   finished feed samples for the presence of AFB$_1$ in Turkey between 1998 and 2000. Samples

149   were collected at farm level, feed mills or imported feeds. In this study, AFB$_1$ was found

150   below detection limits in 43 samples (25.2% of samples), in 20 samples (11.7% of samples)

151   AFB$_1$ levels were above 20 µg kg^{-1} and in 85 samples (50% of samples) AFB$_1$ ranged between

152   21.2 to 42.4 µg kg^{-1}. Authors from this study concluded that levels of AFB$_1$ were higher in

153   samples taken from farms compared to feed mill or imported feed samples.

154   Fegan and Spring (2007) reported, to our knowledge, the first and most complete mycotoxin

155   occurrence survey on fish and shrimp feeds before 2012. Samples were collected in India

156   and Thailand and analysed for the presence of AFs, T-2, ZEN and OTA. No information is

157   available on the period of sampling, region area or sample origin (feed mill or farm).

158   Nonetheless, the information reported shows a different contamination pattern between

159   fish and shrimp feeds and also shows co-occurrence of mycotoxins. Out of the nine fish feed

160   samples analysed from Thailand, all samples were contamination predominantly by ZEN, at

161   levels ranging from 36.20 to 118.48 μg kg^{-1}, followed by T-2 (2.6 to 50.03 μg kg^{-1}) and OTA

162   (2.32 to 7.74 μg kg^{-1}) . Also in Thailand, shrimp feed samples (n=7) were contaminated with

163   ZEN and OTA while no data on AFs was available (Table 1). Shrimp feed samples (n=10)

164   collected from India were mostly contaminated with AFs, ranging between 40 and 90 μg kg^{-1}.

165   However, it is important to mention that levels of sensitivity are mycotoxin-specific and

166   therefore although OTA reported levels were in general lower than ZEN, aquatic species are

167   more sensitive to OTA (see Gonçalves *et al.* 2018 for sensitivity levels in aquatic species). In

168   their study, Fegan and Spring (2007) also reported mycotoxin occurrence in the raw

169   materials used to formulate aquafeeds. While the objective of the present review is only to

170   report mycotoxin occurrence in finished feed, it is inevitable and fundamental to highlight

171   the occurrence of mycotoxins (T-2 and ZEN and OTA) in marine ingredients (fishmeal from

172   China, Myanmar, Thailand; fish and shrimp meal from Thailand) which will be further

173   discussed in next sections.

174   An exception to the almost exclusive AF analysis in finished feeds prior to 2012, are the

175   results presented by Martins *et al*. (2008), who analysed 20 samples of fish feed sourced

176   from Portugal for the presence of AFB$_1$, OTA, DON, ZEN and fumonisin B1 (FB$_1$). In this study,

177   no detectable levels of the target mycotoxins were obtained.

178   In the remaining studies shown in Table 1, in which samples were collected in or before 2012

179   (Alinezhad *et al.* 2011; Almeida *et al.* 2011; Gonçalves-Nunes *et al.* 2015), the target

180   mycotoxin analysed in feed was always AFB$_1$. Almeida *et al*. (2011), did not detect AFB$_1$ in

181   the 87 samples of seabass feed collected in Portugal. Interestingly, 35 of the 87 samples

182   analysed were contaminated with *Aspergillus* spp., which highlights that the presence of

183   fungi does not necessarily mean the presence of the toxin and vice-versa. Alinezhad *et al.*

184   (2011), detected levels high concentrations of AFB$_1$ in fishmeal (average = 67.35 μg kg^{-1}). In

185   Brasil, Gonçalves-Nunes e*t al*. (2015), reported the presence of AFB$_1$ ranging from 1.6 to 9.8

186   µg kg^{-1} in samples collected directly at the feed plant.

187

188   Aquafeed samples after 2012

189   From 2012 onwards, the number of peer-reviewed publications and technical articles (not

190   covered in this review) related to the presence of mycotoxins (including not only AFBs) in

191   aquaculture feeds increased considerably. In 2013, Woźny *et al*. (2013) analysed the

192   presence of ZEN in trout feed collected from three farms in November. One of the farms had

193   no detected levels of ZEN while the other two farms had 81.8 ± 25.8 and 10.3 ± 0.9 µg kg^{-1} of

194   ZEN in their feed respectively. The same study also explored the carry-over of ZEN from feed

195   by analysing several rainbow trout (*Oncorhynchus mykiss*) organs for ZEN presence, results

196   that are further explored in next section. Pietsch *et al*. (2013), unveiled the presence of DON

197   (236.18 µg kg^{-1}) and ZEN (63.82 µg kg^{-1}) in common carp (*Cyprinus carpio*) feeds in samples

198   from central Europe. Still in Europe, Nácher-Mestre *et al*. (2015), investigated the

199   occurrence of mycotoxins in Atlantic salmon (*Salmo salar*) and gilthead sea bream (*Sparus*

200   *aurata*) feeds, with respectively, high and low inclusion of plant meals. From the 18

201   mycotoxins analysed, the most representative mycotoxins found were FUM and DON. In

202   Atlantic salmon, from the three types of feeds analysed, levels of DON were 22.4, 19.4 and

203   23.1 µg kg^{-1} and 148, 754 and 112 µg kg^{-1} of FUM respectively. For gilthead sea bream, two

204   samples were found to contain 79.2 and 53.5 µg kg^{-1} of DON, and 6.4 µg kg^{-1} of FUM in only

205   one of the samples. In Argentina, Greco *et al*. (2015) also analysed salmonids feeds. In this

206   study, 28 samples of rainbow trout (*Oncorhynchus mykiss*) feed were sampled at the farms,

207   ranging throughout the feed portfolio for different development stages (starter feed (13

208   samples); grower feed (13 samples); 4 pigmented and 9 unpigmented feed and finisher feed

209   (2 pigmented samples). The authors observed median values of: AFs = 2.82; OTA = 5.26; T-2

210   = 70.08; DON = 230 and ZEN = 87.97 µg kg^{-1}. It was also highlighted that, there was a co-

211   occurrence of at least two out of six mycotoxins in 93% (26/28) of the analysed samples.

212   Gonçalves *et al*. (2018a; 2018; 2017) focused on unveiling the mycotoxin occurrence in plant

213   meals (not reported here) and aquaculture finished feeds in Europe and Southeast Asia. In

214   2014, from January to December, 41 fish and shrimp feed samples were collected from

215   Europe (n = 6 to 10; Croatia and Portugal) and SE Asia (n = 31; Singapore, India, Thailand and

216 Myanmar). Samples were analysed for AFs, ZEN, DON, FUM and OTA (Table 1). Interestingly,

217 a higher occurrence of FUM was found in European samples (average 3419.92 and maximum

218 7533.61 µg kg^{-1}) compared to SE Asia. The remaining mycotoxins showed similar occurrence

219 average and maximum levels for Europe and SE Asia, with mycotoxins being detected in all

220 analysed samples. In this mycotoxin survey (Gonçalves et al. 2018), it was reported that in

221 Europe, 50% of the samples had more than one mycotoxin per sample, and in Asia, 84% of

222 the samples were contaminated with more than one mycotoxin per feed sample.

223 In 2015, analysing the same mycotoxins as in the previous study, Gonçalves et al. (2017)

224 sourced 25 samples of fish and shrimp feeds in Europe (n = 4; Denmark, Austria, Netherlands

225 and Germany) and SE Asia (n = 21; Vietnam, Indonesia, Myanmar). Contrary to samples

226 collected in 2014, the European samples analysed in 2015 showed relatively low mycotoxin

227 contamination, with only DON contamination reaching values up to 20 µg kg^{-1}. In SE Asian

228 samples, contamination was also generally lower when compared to the previous year, with

229 only AFs showing similar contamination levels to 2014 (average contamination of 58 µg kg^{-1}

230 and maximum of 201 µg kg^{-1}). However, the co-occurrence risk increased in both regions.

231 From January to December 2016, Gonçalves et al. (2018a) sampled four shrimp feeds from

232 India and 12 fish feeds from Indonesia, Myanmar, Taiwan and Thailand. Interestingly, the

233 fish and shrimp feeds showed a relatively different mycotoxin contamination pattern,

234 possibly due to the type of raw materials used to manufacture these diets. Fish feed samples

235 showed lower contamination (Table 1), when compared with shrimp feeds. However, a

236 higher number of co-occurring mycotoxins were observed in fish feeds. Shrimp feeds

237 showed a relatively high contamination of DON, with an average contamination level of

238 881.66 and maximum of 2287 µg kg^{-1}.

239 Mycotoxins also represent a big challenge to the increasingly successful aquaculture sector

240 on the African continent. Marijani et al. (2017), analysed mycotoxin occurrence in Nile tilapia

241 (Oreochromis niloticus) and African catfish (Clarias gariepinus) feeds, gathering 16 samples

242 from Kisumu, Kenya, 13 samples from Ukerewe, Tanzania, 10 samples from Kigembe,

243 Rwanda and 13 samples from Jinja, Uganda. Samples were collected from farms (farm-made

244 feeds; n = 14), local feed millers (n = 14) or imported feeds from Israel and India (n = 12).

245 From the 52 samples analysed, Marijani et al. (2017) observed that farm-made feeds were

246 highly contaminated with AF, FUM and DON (Table 1). On the other hand, feed samples from

247 local feed millers, as well as the imported feed samples, had only minor contamination of AF.

248

249   Discussion on the occurrence of mycotoxins in aquafeeds

250   From the documented peer-reviewed literature, it is possible to observe a growing interest

251   in the occurrence of mycotoxins in aquatic feeds. It is also observable that there is a shift

252   regarding the target mycotoxins analysed in feeds. Most of the earlier studies evaluating

253   mycotoxins in aquafeeds (Bintvihok *et al.*, 2003, Altuğ and Berklevik, 2001) mainly focused

254   on aflatoxin occurrence and only in recent years, other mycotoxins were analysed. This

255   research pattern, i.e., high focus on AFs and only later on other mycotoxins, can also be

256   observed in the peer-reviewed literature studying the impact of mycotoxins in aquatic

257   animal health and performance (Gonçalves *et al.* 2018). The increasing interest in

258   mycotoxins in aquafeeds, and particularly the interest in other mycotoxins besides AFs, is

259   certainly related to the increasing inclusion levels of plant meals in aquafeeds, as well as, the

260   awareness of mycotoxins conveyed from these plant meals to aquafeeds. However, we

261   cannot exclude the easier access to analytical instrumentation to determine mycotoxins

262   together with the evolution of the analytical methods *per se* as a plausible contribution to

263   this shift.

264   The results of the most recent mycotoxin occurrence surveys of aquaculture feeds

265   (Gonçalves *et al.* 2018a; Gonçalves *et al.* 2018; Gonçalves *et al.* 2017; Marijani *et al.* 2017;

266   Nácher-Mestre *et al.* 2015) clearly show an increase in mycotoxin occurrence compared to

267   previous surveys (Alinezhad *et al.* 2011; Almeida *et al.*, 2011; Altuğ and Berklevik, 2001;

268   Bintvihok *et al.* 2003). Unfortunately, it cannot be concluded, from this data, that there is a

269   higher mycotoxin risk now compared to the past. This is because the target mycotoxins

270   analysed in older studies were not the same and sensitivity detection levels and

271   methodologies have since improved significantly. Nonetheless, it was theoretically expected

272   that an increasing level of plant meals in aquafeeds would lead to increased occurrence of

273   mycotoxins in these feeds, which is observable by the most recent occurrence surveys

274   (Gonçalves *et al.* 2018; Gonçalves *et al.* 2018; Gonçalves *et al.* 2017; Marijani *et al.* 2017;

275   Nácher-Mestre *et al.* 2015).

276   Besides the increasing mycotoxin occurrence and the focus on a broad range of mycotoxins,

277   several other important conclusions can be taken from the studies summarized in Table 1. A

278   key aspect is the regional differences in mycotoxin occurrence reported and the correlation

279   between fungi contamination and the presence of mycotoxins. The presence of molds in a

280   fish feed is the first indication that something is wrong with its hygiene. There are several

281   reasons why feeds get moldy, from improper storage conditions (high humidity, high

282   variations in temperatures leading to condensation, etc) to poor manufacturing process

283   (e.g., insufficient drying time, lack of perservatives/anti-molds, etc). Fungi contamination can

284   also originate from inappropriate selection of ingredients, which can carry fungi spores that

285   are resistant to extrusion/pelleting, having the capacity to germinate afterwards (due to

286   improper storage or poor manufacturing processes).

287   While the presence of fungi might be a direct risk for the host, e.g., *Fusarium oxysporum* and

288   *Fusarium solani*, known as opportunistic pathogens for fish and shrimp (Hatai *et al.* 1986;

289   Lightner, 1996; Ostland *et al.* 1987; Souheil *et al.* 1999), and an indirect risk which reduces

290   the palatability and therefore intake of the feed, its presence does not necessarily correlate

291   with the presence of the toxin producer mold and *vice-versa* (Alinezhad *et al.* 2011; Greco *et*

292   *al.* 2015). On the other hand, mycotoxins produced on crops in the field will remain in raw

293   materials, even after processing, due to their heat stability (Pitt, 2014), while fungi will be

294   destroyed due to high temperatures. For example, *Fusarium spp*. are field fungi usually

295   lacking the ability to grow on dry feed. However, the toxins produced by these fungi species

296   (e.g., DON, FUM) will remain stable on the plant raw materials used to manufacture

297   aquafeeds, and in some cases, even be redistributed and concentrated in certain milling

298   fractions (Cheli *et al.* 2013) e.g, corn *vs* corn gluten meal (Gonçalves *et al.* 2018a). Mycotoxin

299   redistribution and transfer from crops to aquafeeds has been observed and reported by

300   Gonçalves *et al*. (2018a). While it is not the core of the present review, we need to highlight

301   that, with the exception of AF and OTA, most of the other mycotoxins found in the

302   occurrence surveys and shown in Table 1 are probably due to the use of plant meals rather

303   than mycotoxins being produced during storage. So, the selection and analysis of the plant

304   raw materials selected to manufacture aquafeeds is the first step to minimise mycotoxin

305   accumulation risks in aquafeeds.

306   The regional differences in mycotoxin occurrence is also an important factor which cannot

307   be overlooked. Fungal growth, and consequently mycotoxin production in crops, is

308   influenced by several factors, with weather conditions being the most important (Miraglia *et*

309   *al.* 2009; Paterson and Lima, 2010; Paterson and Lima, 2011). Consequently, it could be

310   expected that different regions present differences in mycotoxin contamination patterns,

311   and even within a region, mycotoxin occurrence may vary depending on seasonal conditions.

312   This is shown by the data reported by Bintvihok *et al*. (2003) in samples from Thailand, which

313   suggests that rainy seasons might be more problematic and therefore should be closely

314   monitored. However, factors such as climate change and the world trade of commodities

315   makes it challenging to estimate the risk of mycotoxins in aquaculture finished feeds. For

316   example, as reported by Gonçalves *et al* (2018), higher levels of FUM in European finished

317   feeds compared to SE Asia samples cannot be easily explained and therefore a better

318   understanding on the origin of sourced ingredients is necessary. The increasing globalisation

319   of trade commodities and incorporation of imported raw materials into aquafeeds exposes

320   the industry to the potential risk of mycotoxins, which are sometimes not even common for

321   the region (not the case in that particular study). Therefore, mycotoxin contamination needs

322   to take into account the globalisation of raw materials, which could already have significant

323   levels of mycotoxins together with the monitoring of finished feeds.

324

325   **EMERGING MYCOTOXINS**

326   Emerging mycotoxins are a class of mycotoxins which its occurrence in feed and food

327   commodities has been increasing only recently (Kovalsky *et al.* 2016) and which may

328   represent a potential toxicity towards animals and humans. The presence of these

329   mycotoxins also produced by *Fusarium* spp. (as are DON, FUM and ZEN described previously)

330   is expected to increase due to climate change (Miraglia et al. 2009; Paterson and Lima, 2010;

331   Paterson and Lima, 2011). However, quantitative estimates of their occurrence are scarce,

332   especially in aquaculture feeds. While for trichothecenes, data on its toxicity, occurrence,

333   and contamination levels are available, reported in previous section, for other metabolites

334   also produced by *Fusarium* spp., such as moniliformin (MON), fusaproliferin (FUS),

335   beauvericin (BEA) or enniatins (ENNs), limited information is available. Moreover, the typical

336   *Fusarium* mycotoxins (DON, FUM and ZEN) are legislated for certain levels in feed

337   commodities, however, for this new diverse group of "emerging toxins" e.g., MON, FUS, BEA

338   and ENNs, legislation is scarce (Kovalsky et al., 2016). Besides that, the effects of these

339   mycotoxins on aquaculture species is still relatively unknown (Gonçalves et al. 2018; Jestoi,

340   2008; Nguyen et al. 2003; Tuan et al. 2003; Yildirim et al. 2000). Generally, is observed that,

341   regulated mycotoxins, i.e., FUM, DON and ZEN occurrence levels in feeds are still higher than

342   these emerging mycotoxins (Kovalsky et al. 2016). However, Tolosa et al. (2013) identified

343   several enniatins (ENNs; ENA1, ENB and ENB1) in seabream, seabass, tilapia and panga

344   tissues from commercialized aquaculture fishes. To our knowledge, Tolosa et al. (2013) study

345   is the first of its kind and highlights for the need to better understand mycotoxin carry-over

346   beyond the typical *Fusarium* spp. mycotoxins. This topic will be further discussed in section

347   "Data obtained from commercially sourced aquaculture products".

348

349   **CARRY-OVER OF MYCOTOXINS**

350   Bioaccumulation of mycotoxins from feed to animal food products might represent a direct

351   risk to human health (CAST 2003). Mycotoxin bioaccumulation in livestock is well

352   investigated (I. Völkel *et al.* 2011; Leeman *et al.* 2007) and the risk to humans is currently

353   being evaluated by the European Food Safety Authority (EFSA) for several mycotoxins (AF,

354   OTA, ZEN, DON, FUM, T-2 and HT-2). Bioaccumulation of mycotoxins in poultry, swine and

355   cows is managed by direct regulation of mycotoxins in animal feed (EC, 2006; EFSA, 2004a;

356   EFSA, 2004d; EFSA, 2004c; EFSA, 2005; EFSA, 2011; EFSA, 2013). While regulatory limits have

357   been put in place for AFs (), only guidance values are available for DON, OTA, FUM and

358   zearalenone (ZEN; EC, 2006). This is because feed does not represent a direct risk for human

359   health and because carry-over of these mycotoxins in terrestrial animals is expected to be

360   low (EC. 2006).

361   Currently, no regulations or guidelines exist in order to avoid deposition of mycotoxins in

362   farmed fish or shrimp, with the exception of fumonisins (FB1 + FB2 = 10 mg kg^{-1}; EC. 2006).

363   Moreover, it is not taken into consideration that carry-over mechanisms in aquaculture

364   farmed species might be different from terrestrial livestock species. Generally, the possibility

365   of mycotoxin bioaccumulation/biomagnification through the food chain due to the use of

366   mycotoxin contaminated non-plant origin ingredients such as animal by-products (e.g.,

367   shrimp head meal or chicken droppings (further discussed in section "Carry-over data

368   obtained from feeding trials"; "Aflatoxins")) or non-typical mycotoxin contaminated

369   ingredients (e.g., fishmeal), is not taken into consideration and will be addressed during this

370   review.

371   Bioaccumulation of mycotoxins in aquaculture seafood products is not widely reported and

372   consequently not regulated. This section will focus on documented peer-reviewed

373   mycotoxin carry-over studies focussed in aquaculture species. Existing literature is reviewed,

374   calculating transfer factors when the available data allows it, in order to compare

375   bioaccumulation risks (Leeman *et al.* 2007). The transfer factor is expressed as the

376   concentration of mycotoxin in animal tissues ($\mu g\ kg^{-1}$) divided by the concentration of the

377   same mycotoxin in animal feed ($\mu g\ kg^{-1}$).

378

379   Carry-over data obtained from feeding trials

380   The present section intends to give an overview of studies reporting the carry-over of

381   mycotoxins from feed to animal tissues, assessed in feeding trials with supplemented

382   mycotoxins in feed. We calculated transfer factors for carry-over of mycotoxins from feed to

383   eggs, whole milk, meat and edible offal as calculated by Leeman *et al*. (2007) (Table S1). The

384   data presented by Leeman *et al*. (2007) covered 250 references resulting in a comparison of

385   3624 transfer factors from livestock species (cattle, poultry, pig, sheep, goat, rabbit,

386   pheasant, turkey, duck and quail). These authors took into account the carry-over of AFs

387   (AFB$_1$, AFB$_2$, AFG$_1$ and AFG$_2$), DON, OTA, T-2 and ZEN. Leeman *et al*. (2007) reported average

388   transfer factors, ignoring the differences in different mycotoxin kinetics as well as the

389   different metabolism capacity of animals. Nonetheless, the information gathered has a high

390   relevance and allows a first comparison between transfer factors in aquaculture-farmed

391   species *versus* livestock.

392   *Aflatoxins (AFs)*

393   Aflatoxin bioaccumulation from feed to animal tissues is well documented for aquaculture

394   species. A total of 19 studies have evaluated the presence of AFs in fish and crustacean

395   tissues after being fed a certain amount of this same mycotoxin (Table 2).

396   The first study (Suzy *et al.* 2017) reported in Table 2 raises an interesting and not yet

397   discussed point about the occurrence of mycotoxins in feed conveyed from animal by-

398   products and not necessarily from plant meals. Suzy *et al.* (2017) reported that with

399   increasing aquaculture production in Africa, in this case the West Cameroon region, feed

400   ingredients are a serious limitation to the sustainable growth of the aquaculture sector. The

401   author reported that due to the good protein content, chicken droppings were being used as

402   an ingredient in the local fish food or as direct feed, despite its contamination with AF's. Suzy

403   *et al.* (2017) reported that after feeding African sharptooth catfish (*Clarias gariepinus*) with

404   10, 17 and 20 μg AFB$_1$ kg^{-1}, for three months, 0.05 ± 0.12, 0.08 ± 0.10 and 0.08 ± 0.12 μg

405   AFB$_1$ kg^{-1} of AFB$_1$ were found in muscle tissue samples respectively. Calculated transfer

406   factors (0.004 -0.005) (Table 2) for AF in the muscle are within range to values reported for

407   eggs and meat (Leeman *et al.* 2007).

408   Regarding cold/temperate water reared species, five studies are available; in European

409   seabass (*Dicentrarchus labrax*) (El-Sayed and Khalil, 2009)), hybrid sturgeon (*Acipenser*

410   *ruthenusx A. baeri*) (Rajeev Raghavan *et al.* 2011), walleye fish (*Sander vitreus*) (Hussain *et al.*

411   1993) and rainbow trout (*Oncorhynchus mykiss*) (Ellis *et al.* 2000; Ngethe *et al.* 1992; Ngethe

412   *et al.* 1993)) (Table 2). Studies in rainbow trout so far have used tritium (^{3}H) to label AFB_1

413   and it has been not possible to obtain the amount (in μg kg^{-1}) of AFB_1 in tissues. Both

414   authors detected AFB_1 in several samples (faeces, kidney, gastro-intestinal tract, carcass,

415   urine and bile (Ellis *et al.* 2000); bile, liver, kidney, brain, abdominal fat, muscle, spleen and

416   blood (Ngethe *et al.* 1992); liver and brain (Ngethe *et al.* 1993)) up to six (Ngethe *et al.*

417   1993), seven (Ellis *et al.* 2000) and eight (Ngethe *et al.* 1992) days after ingestion of AF. El-

418   Sayed and Khalil (2009), after feeding seabass with 18 μg kg^{-1} of AFB_1, detected 4.25 ± 0.85

419   μg AFB_1 kg^{-1} in muscle samples, which correspond to a TF of 0.278, which is higher than that

420   observed for livestock meat (Table S1). Reported values in muscle in this study (4.25 ± 0.85

421   μg AFB_1 kg^{-1}) are considerably high if one considers that the regulatory limit for AFB_1 in

422   human foods set by the US Food and Drug administration is 5 μg kg^{-1}. Also, in walleye fish

423   (*Sander vitreus*), Hussain *et al.* (1993) reported high levels of AFB_1, AFB_2, AFG_1 and AFG_2 in

424   muscle, which generated TF of 0.1 to 0.5, which are comparable to what is obtained for

425   edible offal and higher than that observed for livestock meat (Table S1). In the case of the

426   Hybrid sturgeon (*Acipenser ruthenusx A. baeri*), animals fed with 40 μg AF kg^{-1} feed, showed

427   values of 28 μg kg^{-1} of AF in muscle and 142.80 μg kg^{-1} in the liver (TF = 0.7 and 3.57)

428   (Raghavan et al., 2011) while when fed with 80 μg kg^{-1} AF the TF were lower both in muscle

429   and liver (TF = 0.4 and 1.15).

430   Tropical species have been particularly studied covering both Asian and South American

431   species. Regarding Nile tilapia (*Oreochromis niloticus*) eight studies have been published to

432   date (Abdel Rahman *et al.* 2017; Ayyat *et al.* 2013; Deng *et al.* 2010; Hessein *et al.* 2014;

433   Hussain *et al.* 2017; Mahfouz and Sherif, 2015, Salem *et al.,* 2009; Selim *et al.* 2014). All

434   studies detected bioaccumulation of AF in muscle and the liver (Table 2). However, these

435   studies vary in terms of fed mycotoxin levels as well as tilapia development stages. Mahfouz

436   and Sherif (2015), used tilapias with an initial weight of 35 ± 0.50 g, and fed them with 20 or

437   100 μg kg^{-1} AF for 12 weeks, with intermediary sampling at six weeks (Table 2). This study

438 found that both AF levels led to accumulation in the liver and muscle, however, in the liver,

439 AFs were found earlier (six weeks post-intake) than in the muscle (only after 12 weeks). The

440 intake period is an important factor to take into consideration as shown by Mahfouz and

441 Sherif (2015), and equally important would be to establish suitable depuration periods for

442 the different mycotoxins. If feasible, adequate fasting periods before harvesting which

443 currently vary from species to species could be set according to mycotoxin tissue levels.

444 Despite using a considerably high range of AFB_1 levels in his study, Deng *et al*. (2010)

445 observed during a 20 week trial, that even relatively low AFB_1 levels (85 μg kg^{-1}) could lead to

446 a significantly high accumulation of AFB_1 in the liver after 20 weeks of ingestion (AFB_1 in the

447 liver after 20 weeks = 30 μg kg^{-1}; Table 2). In short exposure periods to AF (30 days), Abdel

448 Rahman *et al*. (2017) observed that the intake of 200 μg kg^{-1} of AF accumulated in the liver

449 and muscle at 5 ± 0.5 and 3.7 ± 0.1 μg kg^{-1}, respectively. This might suggest a certain

450 incapability to metabolize AF.

451 Other studies also performed in tilapia (*Oreochromis niloticus*) (Ayyat *et al.* 2013; Salem *et*

452 *al.* 2009; Selim *et al.*, 2014), support the previously reported studies, but show a tendency

453 for a higher accumulation of AFs in muscle (Table 2), which could be related to the smaller

454 size of the tilapias used (7 to 15 grams). For example, Selim *et al*. (2014) reported the

455 deposition of 90 μg kg^{-1} of AFs in the muscle after feeding tilapia (15 ± 2 g) with 200 μg kg^{-1}

456 of AF for ten weeks. Likewise, the Ayyat *et al*. (2013) and Salem *et al*. (2009) studies that

457 used fish with an initial weight of 7.3 g and 10 g, respectively, also showed high values of AFs

458 in the muscle (78.33 μg kg^{-1} and 99.48 μg kg^{-1}, respectively). In comparison, in the study by

459 Mahfouz and Sherif (2015) that used fish with an initial weight of 35 g, intake of 100 μg kg^{-1}

460 AF over 12 weeks led to a lower accumulation of AF in the muscle (0.05 μg kg^{-1}). This

461 tendency for higher AF deposition in younger animals seems to be further confirmed by

462 Hessein *et al*. (2014), where after feeding tilapias of 7.3 grams for 98 days with 250 μg kg^{-1}

463 AF, an AF deposition of 101.7 μg kg^{-1} was found. This means a TF of 0.407 that, together with

464 data reported by previous authors (Salem *et al*. 2009, Selim *et al*. 2014), have relatively high

465 TFs for muscle and are only comparable to livestock edible offal (Table S1).

466 Finally, Hussain *et al.* (2017) showed a high deposition of AF in tilapia muscle, however, the

467 levels of mycotoxins used in this trial (2000 to 4000 μg kg^{-1}) are unlikely to be found in

468 aquafeeds although TFs calculated for AF deposition in the liver are in line with the other

469 studies. The only trial with red tilapia (*Oreochromis niloticus x O. mossambicus*), (Usanno *et*

470   *al.* 2005) reported no detectable levels of AF in tilapia tissues, after being fed AF levels

471   ranging from 50 to 2500 µg kg^{-1}.

472   The deposition of AFs in the liver and muscle of Gibel carp (*Carassius gibelio*) are similar to

473   the levels reported for Nile tilapia (Huang *et al.* 2011).

474   Lopes *et al*. (2009) reported the deposition of AFs in the liver and muscle in Jundiá (*Rhamdia*

475   *quelen*) fed low (41.90 and 204 µg kg^{-1}) and high (350, 757 and 1177 µg kg^{-1}) AF levels for 45

476   and 35 days, respectively. Focusing on lower AF levels, as they are whithin the observed AF's

477   occurrence levels in aquafeeds, 41.90 µg AF kg^{-1} feed led to the deposition of 1 µg kg^{-1} in the

478   muscle and 204 µg kg^{-1} of AFs led to the deposition of 6.1 µg kg^{-1} AFs. These bio-

479   accumulation level of AFs leads to TFs of 0.02, which is comparable to the level of

480   accumulation on livestock edible offal's ((Leeman et al. 2007); Table S1)

481   Lambari fish (*Astyanax altiparanae*), a native central/south American small fish (10-15 cm

482   length and 60 g), has been seen as a potential aquaculture species for rural population in

483   Brasil. Michelin *et al*. (2017) reported lambari fish as highly prone to AF deposition in the

484   liver and muscle. After lambari fish were fed 20 kg^{-1} of AFs for 120 days, deposition of AFs in

485   the liver was 265 µg kg^{-1} (TF 13.5) and in fish fed 50 µg kg^{-1} AFs levels in the liver were 243

486   µg kg $^{-1}$ (TF 4.86). This level of bio-accumulation in the liver is higher than the

487   bioaccumulation of highly liposoluble mycotoxins in terrestrial animal fat ((Leeman *et al.*

488   2007); Table S1). Such AFs levels in this species could be particularly challenging as these fish

489   are normally eaten as snacks, i.e., the entire fish is deep-fried, dried and/ or salted.

490   Reports of AF carry-over in shrimp are limited to three studies performed in black tiger

491   shrimp (*Penaeus monodon*). Two of these studies (Bintvihok *et al.* 2003; Bautista *et al.* 1994)

492   did not find any AF residues after feeding shrimps with different AF concentrations (5 to 200

493   µg kg^{-1}) for 10 and 62 days, respectively. In contrast, Boonyaratpalin *et al*. (2001) found AF

494   residues in cephalothorax and in muscle, after feeding the shrimps AFB$_1$ levels ranging from

495   50 to 2500 µg kg^{-1} with TF values ranging from 0.006 to 0.052. Contextualizing the AF

496   contamination levels found in feed around SE Asia (< 500 µg kg^{-1}; (Fegan and Spring, 2007;

497   Gonçalves *et al.* 2018a; Gonçalves *et al.* 2018; Gonçalves *et al.* 2017)) with the

498   Boonyaratpalin *et al*. (2001) study, shrimps fed AFB$_1$ levels of 50 and 100 µg kg^{-1} led to

499   considerably high AF deposition in head and shell (2.6 and 3.5 µg kg^{-1} AFB$_1$, respectively) and

500   in muscle (13 and 14.2 µg kg^{-1} AFB$_1$, respectively), after four weeks of AFB$_1$ intake. For the

501   same intake amounts (50 and 100 µg kg^{-1} AFB$_1$), AFB$_1$ deposition levels in head/shell and

502   muscle samples decreased over time (after six weeks; Table 2). This might suggest a certain

503   capacity to eliminate or metabolize AFB_1.

504

505   *Ochratoxins (OTA)*

506   Ochratoxin bioaccumulation studies in aquaculture-farmed species are very scarce. The most

507   comprehensive study was carried out by Bernhoft *et al*. (2017) in Atlantic salmon (*Salmo*

508   *salar*). Bernhoft *et al*. (2017) studied the deposition of OTA in liver, muscle, kidney and skin

509   samples after feeding salmon with 800 or 2400 $\mu g\ kg^{-1}$ of OTA for eight weeks. Deposition of

510   OTA in kidney and skin samples was not detected (except in kidney for high intake dosage

511   after eight weeks, Table 3). In muscle samples, OTA levels were under the limit of

512   quantification. Major deposition was observed in the liver, however, a bioaccumulation over

513   the exposure period was not found, with the highest OTA deposition peaking after three

514   weeks (both for ingestion of 800 and 2400 $\mu g\ kg^{-1}$ OTA). This suggests that Atlantic salmon

515   might have the ability to eliminate OTA. Previously, OTA deposition in salmonids (rainbow

516   trout (*Oncorhynchus mykiss*)) was investigated by Fuchs *et al*. (1986) where the deposition of

517   OTA in several organs (Table 3) was analysed up to eight weeks after an intravenous

518   injection of OTA (0.160 $\mu g\ kg^{-1}$). Authors observed that OTA deposition in the kidney and bile

519   was persistent during the whole trial, also suggesting the action of the kidney in

520   detoxification mechanism of OTA. The only study reporting carry-over of OTA in shrimp

521   (*Penaeus monodon*) was by Supamattaya *et al*. (2005a), which did not detect OTA deposition

522   in tissues after feeding shrimps with OTA levels ranging from 100 to 1000 $\mu g\ kg^{-1}$. However,

523   the limit of detection given in the manuscript (44,000 $\mu g\ kg^{-1}$) seems to be particularly high

524   for HPLC, suggesting a possible error in the units reported.

525

526   *Deoxynivalenol (DON) and fumonisins (FUM)*

527   Deoxynivalenol and/or FUM bioaccumulation data in aquaculture species is summarized in

528   Table 4. Similar to OTA, DON and FUM carry-over effects in aquaculture-farmed are scarce.

529   In Atlantic salmon (*Salmo salar*), two studies are available (Bernhoft *et al.* 2017 and Nácher-

530   Mestre *et al.* 2015). Bernhoft *et al*. (2017) fed salmon with 2000 and 6000 $\mu g\ kg^{-1}$ DON over

531   the course of eight weeks and sampling liver, muscle, kidney and skin at three, six and eight

532   weeks. The authors observed that both exposure dosages (2000 and 6000 $\mu g\ kg^{-1}$ DON) led

533   to DON deposition in the liver and muscle at all sampling points, except for the higher

534   dosage at the last sampling point (eight weeks), at which DON was found in all sampled

535   tissues (Table 4). In the case of the study performed by Nácher-Mestre *et al*. (2015), Atlantic

536   salmon were fed lower levels of mycotoxins, however, with multi-occurrence. The three

537   diets were mainly formulated with DON and FUM, but also minor levels of T-2 and 15-

538   AcDON (Table 4). Salmon fed for six months with testing diets did not show detectable levels

539   of DON and FUM in the tissues studied. The same authors (Nácher-Mestre *et al.* 2015) also

540   studied bioaccumulation of mycotoxin co-occurrence (DON, 15-AcDON and FUM) in Gilthead

541   sea bream (*Sparus aurata*) at two levels for 8 months. The authors did not observe

542   mycotoxin deposition in muscle samples.

543   In common carp (*Cyprinus carpio*), Pietsch *et al.* (2014) observed that after feeding fish with

544   352, 619 and 953 µg kg^{-1} DON for four weeks, minor deposition of DON was observed in the

545   muscle (Table 4). Interestingly, after the four weeks of DON exposure, fish were fed a non-

546   contaminated diet for a period of two weeks and DON levels in the muscle were re-analysed.

547   At the lower DON intake level (352 µg kg^{-1}), DON level in the muscle was higher after the

548   depuration period (1.4 µg kg^{-1}) when compared to the level found at the end of feeding trial

549   (eight weeks; 0.6 µg kg^{-1} DON). At the medium DON intake level (619 µg kg^{-1}), after the

550   recovery period, a level of 0.7 µg kg^{-1} DON was still found in the muscle, and at the higher

551   level, however, no DON was detected after the recovery period.

552   In shrimps, two studies are available (Supamattaya *et al.* 2005b and Trigo-Stockli *et al.* 2000)

553   Table 4), in which both reported that DON was not detected in the muscle. Supamattaya *et*

554   *al.* (2005b) drew its conclusion after feeding black tiger shrimp black (*Penaeus monodon*)

555   with 500, 1000 and 2000 µg kg^{-1} DON for eight weeks. Trigo-Stockli *et al.* (2000) conducted

556   its study using Pacific white shrimp (*Litopenaeus vannamei*), fed with 200, 500 and 1000 µg

557   kg^{-1} DON for 16 weeks.

558

559

560   *Zeralenone (ZEN)*

561   Zearalenone (ZEN) is a regular contaminant of cereal crops worldwide, and being a

562   phytoestrogenic compound (Diekman and Green, 1992), is mainly responsible for

563   estrogenic agonist related effects (Marasas, 1991). As a hormone mimicking substance, ZEN

564   can bind to estrogen receptors in target cells (Kumar *et al.,* 2013). Generally, ZEN studies

565   have focused mainly on dysfunction or structural disorders in the reproductive tract of farm

566   animals (Minervini and Aquila, 2008; Zinedine *et al.* 2007; Woźny *et al.* 2013). While it seems

567   that ZEN does not directly affect the growth performance of aquaculture-farmed species, its

568   deposition in fish tissues seems to be common and already well documented particularly in

569   cold water species (Pietsch *et al.* 2015; Woźny *et al.* 2015; Arukwe *et al.* 1999; Woźny *et al.*

570   2017).

571   In common Carp (*Cyprinus carpio*), Pietsch *et al.* (2015) found that after exposing fish to four

572   weeks with 332, 621 and 797 µg ZEN kg^{-1} feed, minor residues of ZEN and α-ZEN were found

573   in the muscle. Interestingly, after two weeks of depuration, α-ZEN was not detected and ZEN

574   levels in the muscle decreased significantly (Table 5).

575   Woźny *et al.* (2015; 2017) dedicated significant efforts at understanding the potential of ZEN

576   bioaccumulation in fish, using mainly rainbow trout as a model. The authors found that after

577   feeding rainbow trout with 1,810 µg ZEN kg^{-1} feed for 71 days, ZEN was found at a

578   concentration of 732.2 µg kg^{-1} in the intestine while non-quantifiable levels of ZEN were

579   found in liver and female ovaries. In another trial, Woźny *et al.* (2017) used mature females

580   (1,274 ± 162 g) to study ZEN carry-over into eggs. Authors found that ZEN is transferred from

581   the gastrointestinal tract to the reproductive system of the fish, depositing ZEN metabolites

582   in the somatic cells of the ovaries rather than in the oocytes.

583

584   Discussion on the carry-over data obtained from feeding trials

585

586   In order to take realistic conclusions regarding the risk of mycotoxin consumption from

587   aquaculture seafood products, it is necessary to have a good overview of mycotoxin

588   occurrence in aquaculture feeds, and to have quality data on mycotoxin bioaccumulation in

589   aquatic species.

590   From all the studies regarding AF carry-over presented in Table 2, a few of them should be

591   excluded due to the use of high levels of AFs (Hussain et al. 2017); or higher dosages, which

592   are not normally observed in commercial feeds (Deng et al.,(2010), Boonyaratpalin et al.

593   (2001) and Usanno et al. (2005)). The studies reported by the remaining authors, employed

594   plausible dietary mycotoxin levels, identifying the carry-over of AFs in several important

595   species.

596   From these studies, it is possible to conclude that AFs might represent a serious risk for

597   human consumption, especially in cases where fish are eaten as a whole. In general, transfer

598   factors are quite high for these aquaculture species, being comparable with transfer factors

599   for eggs, whole milk and in some cases for edible offal's or fat of livestock provenience.

600   In the case of European seabass, mycotoxin levels tested by El-Sayed and Khalil (2009) (18 µg

601   kg^{-1}), which is a mycotoxin level very plausible to be obtained in commercial diets led to 4.25

602   ± 0.85 µg AFB$_1$ kg^{-1} in the muscle,. As shown by Altuğ and Berklevik (2001) (Table 1), of the

603   170 samples collected in Turkey, which is the main EU seabass producer, 105 samples were

604   contaminated with AFs at levels higher than 20 µg kg^{-1}. Regarding hybrid sturgeon (*Acipenser*

605   *ruthenus*), there is no available mycotoxin occurrence data for this species, even in regions

606   where it is predominantly produced. However, in-feed concentrations tested by Rajeev

607   Raghavan *et al*. (2011), which led to the accumulation of AF in the muscle and liver, seem

608   realistic (40 to 80 µg AFB1 kg^{-1}) and therefore further research should be carried out to

609   determine mycotoxin levels in feed for this species and AF accumulation in eggs (caviar).

610   Carry-over effects on Nile tilapia are well described. Taking into account the available

611   occurrence of AF in tilapia producing countries, i.e., Brasil (Barbosa *et al.* 2013), S/ SE Asian

612   countries (Fegan and Spring, 2007; Gonçalves *et al.* 2018a; Gonçalves *et al.* 2018; Gonçalves

613   *et al.* 2017) and Africa (Marijani *et al.* 2017) together with bioaccumulation studies, carry-

614   over of AF in Nile tilapia might represent a challenge worth of further investigation. From

615   the previously cited studies, it is also important to highlight that exposure period is an

616   important factor to take into consideration. Chronic exposure to low AF levels (AF = 85 µg

617   kg^{-1} for 20 weeks) could lead to a significantly high accumulation in the liver (AF in the liver

618   after 20 weeks = 30 µg kg^{-1} (Deng *et al.* 2010)). However, short exposure periods should not

619   be undervalued, as periods as short as 30 days can lead to considerable AF deposition in the

620   liver and muscle (Abdel Rahman *et al.* 2017).

621   Aflatoxin carry-over studies in shrimp are more limited than in fish species. Furthermore, the

622   information available is contradictory, as two studies (Bintvihok *et al.* 2003 and Bautista *et*

623   *al.* 1994) did not find any AF residues in tiger shrimp muscle while Boonyaratpalin *et al.*

624   (2001) found AF bioaccumulation in head/shell and in the muscle. Results suggested a minor

625   bioaccumulation over time (TFs; Table 2), highlighting a certain capacity to eliminate or

626   metabolize AFB$_1$. However, levels of AF found in the muscle (13 µg kg^{-1} AFB$_1$) after feeding

627   shrimps 50 µg kg^{-1} of AFB$_1$ for four weeks were considerably high and could be a threat for

628   human food safety. AF deposition, especially in head samples, should not be undervalued. In

629   many countries, heads are used for direct human consumption. Unfortunately, no

630   information is available for Pacific white leg shrimp (*Litopenaeus vannamei*) which is the

631   most important produced shrimp species in terms of volume.

632   For OTA occurrence, little information is available for aquaculture feeds, however, according

633   to available studies, levels below 10 µg kg^{-1} have been reported (Fegan and Spring, 2007;

634   Gonçalves et al. 2018a; Gonçalves et al. 2018; Gonçalves et al. 2017; Greco et al. 2015). The

635   risk of OTA carry-over was only successfully addressed in Atlantic salmon and partially in

636   rainbow trout. In Atlantic salmon (Bernhoft *et al.* 2017), it would appears that OTA is rapidly

637   eliminated. Its deposition in tissues was only shown in liver (4.81 µg kg^{-1}) and only at the

638   highest OTA intake level (2400 µg kg^{-1}). These OTA levels are unlikely to be observed in

639   commercial feeds. In rainbow trout, OTA deposition in the muscle was not detected after

640   24h of OTA intake. This again suggests a rapid elimination of OTA and decreases the risk for

641   human consumption as fasting periods before slaughter in salmonids are normally longer

642   than 24 hours. However, it is highly recommended that more studies are undertaken on OTA

643   carry-over, especially for species were OTA occurrence in feeds is more frequent and higher,

644   such as tropical species, where fasting periods before harvest also tend to be much shorter

645   than for cold-water species and also tropical crustacean species.

646   DON, FUM and ZEN occurrence in aquafeeds have been well documented in recent years

647   (Pietsch et al. 2013; Nácher-Mestre et al. 2015; Gonçalves et al. 2018a; Gonçalves et al.

648   2018; Gonçalves et al. 2017; Greco et al. 2015; Marijani et al. 2017). These mycotoxins have

649   been pointed out as the main mycotoxin contaminants in aquaculture feeds, which is a

650   reflection of the increasing inclusion levels of plant meals in diets, as these mycotoxins are

651   produced in field conditions. However, DON and FUM bioaccumulation has been poorly

652   studied   in   aquaculture-farmed   species.   In   Atlantic   salmon,   two   interesting   and

653   complementary studies are available (Bernhoft *et al.,* 2017 and Nácher-Mestre *et al.,* 2015).

654   While Bernhoft *et al.* (2017) proved the possibility of DON deposition in the liver and muscle

655   in a relatively short exposure period (three weeks) with high DON levels (2000 and 6000 µg

656   kg^{-1} DON), Nácher-Mestre *et al.* (2015) showed no carry over effects of FUM and DON co-

657   contamination at low levels during long exposure periods. DON and FUM frequently occur

658   together in aquaculture feeds as both mycotoxins are produced by the same fungi species.

659   Therefore, studies testing the effect of co-occurrence are particularly relevant. The levels

660   tested were within the occurrence values reported in European aquafeeds (Gonçalves *et al.*

661   2017; Gonçalves *et al.* 2018), however, occasional high occurrences of DON and/or FUM

662   should not be ignored (e.g., FUM occurrence reported by Gonçalves *et al.* (2018)), as shown

663   previously, levels up to 2000 µg kg^{-1} can lead to DON deposition in the muscle.

664   Contrary to Atlantic salmon, in common carp (*Cyprinus carpio*), Pietsch *et al.* (2014) showed

665   that levels as low as 352 µg kg^{-1} DON can lead to a minor deposition of DON in the muscle

666   (Table 4). The author described that total DON elimination from the muscle is a relatively

667   long process, taking more than two weeks after stopping DON intake. Information about the

668   complete elimination of DON is very important, as a fasting period before harvesting may be

669   used to guarantee that DON or any other mycotoxin is eliminated during this period.

670   However, in the study reported by Pietsch *et al*. (2014), the elimination period of DON in

671   carp may be longer than the fasting period, which is normally 24 to 48 hours before

672   harvesting. The study by Pietsch *et al*. (2014) highlighted that mycotoxin absorption,

673   distribution, metabolism, and excretion (ADME) is entirely dependent on species, and data

674   or conclusion extrapolations between species should be avoided. *Fusarium* mycotoxins (e.g.,

675   DON and FUM) are frequently present in plant commodities used for general aquaculture

676   species, and taking into account the possible ADME differences depending on species and

677   even on development stages, it would be very important to better understand the potential

678   carry-over in the most important aquaculture species, giving a special emphasis to

679   mycotoxin co-occurrence.

680   Despite the low number of studies on DON and FUM carry-over, apparently, its deposition in

681   tissues seems to be very limited. However, its occurrence is frequent and due to its

682   apparently long elimination period (generally higher than fasting period before slaughter, for

683   the study species), its carry-over risk in aquaculture-farmed species should be better

684   evaluated. Comparing TFs obtained from Atlantic salmon and common carp, it seems that

685   they are in line with the TFs of eggs, whole milk or meat (Table S1, (Leeman *et al.* 2007)).

686   Is also important to highlight that the species investigated so far are cold/temperate water

687   species. It is essential to increase the knowledge on the possible carry-over of *Fusarium spp.*

688   mycotoxins in tropical species. Especially high value species, normally exported, such as

689   Pacific white leg shrimp, whose feeds have been identified recently as being contaminated

690   with considerably high levels of DON (Gonçalves et al. 2018a). Furthermore, these tropical

691   species present a faster metabolism and consequently lower fasting period before harvest is

692   need, which might greatly influence the deposition of mycotoxins in tissues.

693   From the few available studies evaluating ZEN carry-over effects, it is possible to conclude

694   that, at least for the cold-water species studied so far (common carp and rainbow trout),

695   ZEN and its metabolites can be deposited in several tissues, including muscle, intestine, liver,

696   ovaries and oocytes. However, the levels found in these tissues, with the exception of the

697   intestine and liver (Table 5, (Woźny *et al.* 2017)), are rather low and do not pose a direct risk

698   to human consumption. In the European Union, the maximum allowable level of ZEN ranges

699   from 20 µg kg^{-1} for processed cereal-based foods (excluding processed maize-based foods)

700   and baby foods for infants and young, to 300 µg kg^{-1} for unprocessed maize (not for human

701   consumption) (EC, 2006). However, European legislation does not include limits for the

702   concentration of ZEN residuals in food of animal origin, since it is thought that carry-over of

703   the *Fusarium* mycotoxins (including DON and FUM previously discussed) to meat, milk and

704   eggs is only minimal (CONTAM, 2011; EC, 2006).

705   Moreover, ZEN and its metabolites seem to be more easily deposited in the somatic cells of

706   the ovaries rather than in the oocytes. For rainbow trout and common carp, tissues such as

707   ovaries, liver and intestines are not typically edible, however, for other species this might

708   not be the case. It would be very important to assess the carry-over of ZEN and its

709   metabolites for other aquaculture-farmed species, taking into account what is already

710   known in rainbow trout and common carp. It is particularly interesting to evaluate species

711   that reach sexual maturation before or near harvesting size. ZEN in feed may accelerate the

712   sexual maturation of the fish, leading to energy losses to gonad development, and in some

713   cases organoleptic and physical changes of the final product. For some species, ZEN in feed

714   may also have potential implications for fish and shrimp spawning and further studies need

715   to address this topic. In addition, fish/shrimp species that might be consumed entire, i.e.,

716   including tissues such as the liver, intestines and ovaries should be taken into consideration,

717   as ZEN might reach considerably high levels in these tissues. In certain cases, the use of

718   fish/shrimp by-products in direct human consumption (fish oil) or as an ingredient to

719   formulate new products, should also be taken into consideration as *Fusarium* mycotoxins

720   tend to be quite stable to processing conditions and only minor degradation is expected

721

722   Data obtained from commercially sourced aquaculture products

723   Table 6 documents mycotoxin occurrence in commercially sourced aquaculture products.

724   Evaluating the occurrence of mycotoxins directly in fish/shrimp products from aquaculture

725   provenience obtained from commercial farms or local supermarkets is a good strategy to

726   evaluate the potential risk of mycotoxin carry-over from feeds to fish/shrimp edible

727   products. Tolosa *et al.* (2013) analysed several samples (n = 19) of fish from aquaculture and

728   wild fishery provenience bought locally in Spain. The author analysed samples for the

729   presence of beauvericin (BEA) and enniatins (enniatin A (ENA), enniatin A1 (ENA1), enniatin

730   B (ENB) and enniatin B1 (ENB1)). As expected, no mycotoxins were detected in the wild

731   fishery samples. ENA and BEA were also not detected in the aquaculture samples. However,

732   ENA1, ENB and ENB1 were detected in most of aquaculture samples (Table 6). Detecting

733   enniatins in aquaculture foods might lead us to two hypothesis. First, that other *Fusarium*

734   mycotoxins (FUM, DON and ZEN mainly) were probably at even higher concentration levels

735   and are not reported as they were not analysed. The second hypothesis is the fact that ENNs

736   might be more easily deposited in the muscle compared to DON/FUM, even if present at

737   lower levels in aquafeeds. As it is known that ENNs normally occur together with the main

738   *Fusarium* mycotoxins (FUM, DON), it would also be important to study if this synergistic

739   presence in the tissues might lead to increased deposition of certain mycotoxins or

740   metabolites. While it is difficult to evaluate the importance of detecting ENNS in aquaculture

741   foods, these results highlight the need to better study the adverse effects of dietary

742   mycotoxins on fish health and welfare, and consequently carry-over risks. There is the need

743   to perform studies for the main EU farmed fish species in order to establish acceptable feed

744   mycotoxin levels for farmed fish (for both fish and consumer safety), but also to actively

745   survey possible mycotoxin deposition in imported aquaculture foods.

746   Woźny *et al.* (2013) analysed ZEN in rainbow trout from farms based in the north-eastern

747   region of Poland. ZEN was present at non-quantifiable levels (<2.0 µg kg^{-1}) in most of the

748   tissues analysed (intestine, liver and ovary) and detectable at quantifiable levels in the

749   muscle and surrounding water. From 2013 to 2015, Woźny *et al.* (2017) surveyed ovary,

750   oocytes and salted roe samples from different fish species collected directly at hatcheries or

751   bought in supermarkets. The authors analysed the samples for the presence of ZEN, α-ZEL

752   and β-ZEL. Generally, in most of the samples analysed mycotoxins were below the detection

753   limits (LOD for ZEN, α-ZEL, and β-ZEL were 5.0, 3.0, and 12.0 µg kg^{-1}, respectively). The

754   exceptions were α-ZEL in ovary samples (14.5 µg kg^{-1}) of *Oncorhynchus mykiss* and α-ZENL

755 also in ovary samples (12.6 µg kg^{-1}) of *Salvelinus fontinalis* both sampled in 2014. The studies

756 reported by Woźny *et al*. (2013; 2017) are also extremely important and highlight the need

757 for guidance values for the amount of ZEN in aquafeeds for fish health and reproductive

758 performance, but also to avoid carry-over risk to human consumers.

759 Although it did not investigate fish originating from aquaculture, it is important to highlight

760 the recent study published by Slawomir Gonkowski et al., (In Press). Slawomir Gonkowski *et*

761 *al*., (In Press) evaluated the deposition of ZEN in sun-dried kapenta fish, which is one of

762 Zambia's major staple foods. This small planktivorous fish is caught in Lake Kariba, sun-dried

763 and sold in local markets. Although the source of the ZEN deposition is not known, the study

764 revealed that levels of ZEN in sun-dried kapenta fish fluctuated from about 27 µg kg^{-1} to

765 above 53 µg kg^{-1}. Occurrence of ZEN in sun dried kapenta fish, highlights that carry-over

766 guidelines cannot be assumed only for farmed animals as species and local consumption

767 habits pose mycotoxin-related risks to wider seafood products.

768

769 **Further considerations**

770 Despite the effort to document mycotoxin occurrence in aquaculture feeds, we are still far

771 from having a good overview on this topic. One of the big challenges is the large number of

772 aquaculture-farmed species, and the impossibility to extrapolate occurrence results from

773 one species to another. Moreover, different species, even in same trophic level, tend to be

774 fed with different raw materials based on local availability and price. This leads to a huge

775 difficultly in having a good overview of mycotoxin occurrence for all aquaculture species or

776 even for a certain region. Nevertheless, knowledge about mycotoxin occurrence in

777 aquaculture commodities could increase significantly if we could better use the available

778 occurrence data from livestock. Surveys on mycotoxin occurrence in plant meals worldwide

779 are frequently available, and this information can be used, at least, to theoretically model

780 the risk of plant feedstuffs included in aquafeeds. However, a fundamental problem is the

781 lack of detailed labelling information regarding ingredient inclusion by (percentage) weight.

782 Therefore, an improvement in labelling policy would help to identify and map sources of

783 mycotoxin inclusion in animal feed, avoiding extra costs for testing mycotoxin levels in

784 finished feeds. Therefore, a close collaboration with the agricultural and livestock sectors to

785 understand the occurrence of mycotoxins in plant meals, might also help to improve our

786 knowledge on mycotoxin conveyance to aquafeeds.

787   Mycotoxins conveyed from land animals and aquaculture by-products cannot be despised,
788   especially in countries were mycotoxin occurrence might be poorly legislated. The
789   identification of mycotoxins in shrimp head meal or chicken droppings highlights the
790   possible bio-amplification through the food chain.

791   To our knowledge not yet addressed in an aquaculture context, is the potential for
792   mycotoxins to contaminate water, especially taking into account water stable mycotoxins
793   and closed or semi-closed aquaculture systems. Bucheli *et al.* (2008) evaluated the presence
794   of ZEN and DON in Swiss rivers, confirming the presence of both mycotoxins at levels ranging
795   from 23 ng L^{-1} to 4.9 μg L^{-1} for DON and 35ng L^{-1} for ZEN. Bucheli *et al*. (2008) highlighted the
796   possibility of mycotoxins as water contaminates, which in the aquaculture context might be
797   extremely relevant. The mycotoxin leach from aquafeed to system water, especially of highly
798   water-soluble mycotoxins in slow feeding species, e.g., DON and FUM in shrimp feed, and
799   the water stability of excreted mycotoxins and metabolites, which might have potential to
800   accumulate, especially in low water hydrodynamics and low renovation rate aquaculture
801   systems, should be urgently addressed.

802

803   **CONCLUSION**

804

805   The available carry-over studies indicate that deposition of mycotoxins into edible tissues
806   may be higher than in terrestrial species and it is therefore imprudent to assume the same
807   transfer factors for aquaculture species as for livestock species. In general, aflatoxins seem
808   to be particularly prone to deposition in several fish and shrimp tissues representing a risk
809   for human consumption, especially in species that are eaten as a whole. Ochratoxin A
810   occurrence in aquafeeds has been described as very low. While its deposition in tissues has
811   been reported for some aquaculture species, its rapid elimination decreases the risk for
812   human consumption as the fasting period before slaughter can be safely used as a
813   depuration period. Nonetheless, it is important to make the industry aware of its possible
814   deposition. Deoxynivalenol and fumonisins are some of the most frequently occurring
815   mycotoxins in feeds, and they are occasionally detected at high levels. So far, for the species
816   described, DON and FUM deposition in tissues seems low. However, DON elimination from
817   the muscle takes a relatively long time, much longer than the depuration/fasting period. The
818   presence of enniatins in aquaculture food products highlights the possibility that other

819 *Fusarium* metabolites might be more prone to bioaccumulation than the most common

820 frequently analysed *Fusarium* mycotoxins. The presence of enniatins in aquaculture foods

821 highlights the need to understand its potential impact to human food safety.

822 Regarding ZEN, the potential for deposition in the ovaries and to a lesser extent in oocytes

823 was shown. For the studied species, ZEN can reach considerable levels in the ovaries. No

824 studies are available yet for tropical species. It would be important to investigate whether

825 carry-over of ZEN to ovaries occurs in tropical species as well, as for many of these species,

826 gonads are considered gourmet snacks, representing a direct risk to human health.

827 While there are many important aquaculture species not investigated yet, it is clear that

828 some mycotoxins are prone to deposition in the tissues of certain aquaculture species. It

829 needs to be considered that in aquaculture species, mycotoxin biotransformation and

830 tendency for deposition in tissues varies greatly depending on factors such as development

831 stage, sex, exposure period and rearing environment.

832 Due to the use of increasing levels of plant meals in aquafeeds and together the possible

833 mycotoxin increase due to climate change, it is essential to develop more studies on the

834 impact of mycotoxins and metabolites on farmed species with consequent risk assessment

835 of food safety from mycotoxin-contaminated aquafeeds.

836 Regulation limits for mycotoxins in feeds might need to take into account particular

837 aquaculture species or the sector as a whole. Mycotoxin limits need to take into

838 consideration animal health and welfare but also human health. Particular attention needs

839 to be focused on aquaculture edible tissues and regional guidance limits should be advised

840 depending on local mycotoxin occurrence and the edible tissues consumed. Risk assessment

841 of imported aquaculture foods needs to take into account the mycotoxin occurrence,

842 especially in those products imported from highly mycotoxin contaminated regions, or

843 regions known to use potentially contaminated animal by-products.

844

845

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Table 1: Documented mycotoxin occurrence in aquaculture feeds.

Reference	Sampling year(s)	Sampling Country	Sampling local	Number of samples	Species to which the feed is intended	Target mycotoxin analysed in feed	Mycotoxin detection level ($\mu g\ kg^{-1}$)	Method of analysis	Observations
Bautista et al. 1994	August 1990 - February 1991 (rainy season)	Philippines	Feed plant Farm level	n = 62	Black tiger shrimp	AFB_1	n = 2 -> none detected n = 36 -> 10 to 20 $\mu g\ kg^{-1}$ AFB_1 n = 21 -> 30 to 40 $\mu g\ kg^{-1}$ AFB_1 n = 2 -> 60 to 120 $\mu g\ kg^{-1}$ AFB_1	HPTLC	------
Bintvihok et al. 2003	[S]Summer (March - June 1997) [R]Rainy (July - October 1997) [W]Winter (November - February 1998)	Thailand (Eastern and Southern regions)	Farm level	N_t =150 (50 samples from 10 different regions during 3 seasons)	Black tiger shrimp	AFB_1 AFB_2 AFG_1 AFG_2	Eastern region [S]0.003–0.012 [R]0.003-0.651 [W]0.003-0.314 Southern region [S]0.004 [R]0.003-0.058 [W]0.003-0.022	HPLC	▪ Feeds composed mainly of fishmeal, soybean and corn (no information on ingredient inclusion levels or finished feed storage period)
Altuğ et al. 2001	1998, 1999, 2000	Turkey	Farm level Feed plant Imported feeds	n = 170	Rainbow trout Seabream Pike-perch	AFB_1	n = 20 > 20 $\mu g\ kg^{-1}$ n = 85 = 21.2 to 42.4 $\mu g\ kg^{-1}$ n = 22 = 5.0 to 20.0 $\mu g\ kg^{-1}$ n = 43 < LOD	TLC ELISA	▪ Level of aflatoxins were higher in samples that were taken from farm level compared to feed plant or imported feed samples
Alinezhad et al. 2011	March - July 2009 (1 sample per month)	Iran	Feed plant	n = 6	Rainbow trout	AFB_1	0.12 to 20.09 $\mu g\ kg^{-1}$ AFB_1	HPLC	▪ High concentrations of AFB_1 in fishmeal ($\bar{x}$= 67.35 $\mu g\ kg^{-1}$) and soybean meal ($\bar{x}$= 30.88 $\mu g\ kg^{-1}$)
Fegan & Spring, 2007	n/a	[IN]India [TH]Thailand	n/a	[IN,S]n= 10 [TH,S]n= 7 [TH F]n= 9	Shrimp[S] Fish[F]	[IN,S](AF, T-2, ZEN) [TH,S](T-2, ZEN, OTA) [TH,F](T-2, ZEN, OTA)	[IN,S]AF = 40-90; (9/10) [IN,S]T-2 = 20-40; (4/10) [IN,S]ZEN = 20-40; (4/10) [TH,S]T-2 = 2.6-50.03; (3/7) [TH,S]ZEN = 16.78-23.00; (6/7) [TH,S]OTA = 2.32-7.74; (7/7) [TH,F]T-2 = 15.91-49.13; (9/9) [TH,F]ZEN = 36.20-118.48; (9/9) [TH,F]OTA = 2.16-9.72; (9/9)	n/a	▪ Marine ingredients (fishmeal from China, Myanmar, Thailand; fish and shrimp meal from Thailand) contaminated with T-2, ZEN and OTA
Goncalves-nunes et al. 2015	January - March 2009	Brazil (Piauí State)	Feed plant	n = 18	Fish	AFB_1	1.6 - 9.8	ELISA	▪ Finished feed samples were composed of soybean bran (15%), corn bran (27%), other cereals (57.5%).
Barbosa et al. 2013	September 2009 and August 2010	Brasil (Rio de Janeiro State)		n = 60	n/a	FB_1 AFB_1 OTA	FB_1 = (90%) 0.3-4.94; $\bar{x}$ = 2.6 AFB_1 = present in 55% of the samples OTA = present in 3.3% of the samples	FB_1 - ELISA AFB_1 and OTA - TLC	LOD: ▪ 0.2 $\mu g\ g^{-1}$ for ELISA (FB_1) ▪ 0.003 and 0.005 $\mu g\ g^{-1}$ for TLC (AFB_1 and OTA) ▪ 50% of samples had co-occurrence of

							No levels mentioned for AFB$_1$ and OTA		AFB$_1$ and FB$_1$ ▪ 3.3% of the samples tested positive for the three mycotoxins analysed
Martins et al. 2008	n/a	Portugal	n/a	n =20	Fish	AFB$_1$ OTA DON ZEN FB$_1$	N.d	HPLC	LOD ▪ AFB$_1$ = 0.2 µg kg^{-1} ▪ OTA = 20 µg kg^{-1} ▪ DON = 100 µg kg^{-1} ▪ ZEN = 50 µg kg^{-1} ▪ FUM = 20 µg kg^{-1}
Almeida et al. 2011	n/a	Portugal	Feed plant	n = 87	Seabass	AFB$_1$	AFB$_1$ n.d. (detection limit of the method was 1.0 µg kg^{-1})	HPLC	▪ 35 samples contaminated with *Aspergillus* spp.
Pietsch et al. 2013	n/a	Central Europe	n/a	n = 11	Carp	DON ZEN	DON = 66-825; x̄ = 236.18 ZEN = 3-511; x̄ = 63.82	HPLC	▪ Most common plant ingredients in feeds collected: C = corn; CGF = Corn gluten feed; SEM = soybean extraction meal; SM = soybean meal; SFEM = sunflower feed extraction meal; W = wheat; WB = wheat bran, WDB = wheat distillery by-product; WGF = wheat gluten feed.
Woźny et al. 2013	November 2012	Poland (North-eastern region)	Farm level	n = 3	Trout	ZEN	#$_1$ = n.d. #$_2$ = 81.8 ± 25.8 #$_3$ = 10.3 ± 0.9	HPLC	▪ Rainbow trout organs were also sampled, refer to table 6.
Greco et al. 2015	n/a	Argentina (Río Negro and Neuquén)	Farm level	n =28	Rainbow trout	AF OTA T-2 FUM DON ZEN	AF = 1.3 − 8.91; x̃ = 2.82 OTA = 3.5 − 5.0 x̃ = 5.26 T-2 = 50 − 105.99; x̃ =70.08 FUM = 190 -222; x̃ = -- DON = 150 − 210; x̃ =230 ZEN = 20.04 − 159.76; x̃ = 87.97	ELISA	▪ Finished feed samples were composed of soybean expeller, disabled soybean, corn, wheat, wheat bran, corn gluten meal ▪ Co-occurrence of at least two out of six mycotoxins was recorded in 93% (26/28) of samples analysed
Nacher-Mestre et al. 2015	n/a	United Kingdom	Feed plant	n = 5 2 diets[GSB] with low level plant meal 3 diets[AS] with high level plant meal	[AS]Atlantic salmon [GSB]Gilthead sea bream	AFB$_1$, AFB$_2$, AFG$_1$, AFG$_2$, OTA, NEO, FB$_1$, FB$_2$, FB$_3$, T-2, DIA, ZEN, NIV, DON, 3-AcDON, 15-AcDON, FUX, and HT-2	DON[GSB] = 79.2 and 53.5 DON[AS] = 22.4 , 19.4 and 23.1 FUM[GSB] = -, 6.4 FUM[AS] = 148, 754 and 112	LC–MS/MS	▪ No carry-over effects observed after 8[GSB] and 7[AS] months of feeding the contaminated diets. ▪ Diets manufactured with contaminated ingredients (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).
Gonçalves et al. 2018	January 2014 – December 2014	[A]Asia (CN, IN, TH, MN) [E]Europe	Farm level Feed plant	N$_t$= 41 samples n = 31 Asia	Shrimp Fish	AF ZEN DON FUM	[A]AF: x̄ = 51.83; Max = 220.61; (21/31) [A]ZEN: x̄ = 60.41; Max = 232.88; (18/31) [A]DON: x̄ = 160.86; Max = 413.08;	HPLC	▪ In Europe, 50% of the samples had more than 1 mycotoxin per sample ▪ In Asia, 84% of the samples had more than 1 toxin per feed

Reference	Period	Region (Country)	Source	Sample size	Species	Mycotoxins	Results	Method	Notes
		(CR, PT)		n = 6-10 Europe		OTA	(21/31) [A]FUM: $\bar{x}$ = 172.63; Max = 573.32; (18/31) [A]OTA: $\bar{x}$ = 2.11; Max = 5.05; (17/31) [E]AF: $\bar{x}$ = 0.43; Max = 0.43; (1/6) [E]ZEN: $\bar{x}$ = 118.01; Max = 305.89; (4/6) [E]DON: $\bar{x}$ = 165.61; Max = 281.72 (4/6) [F]FUM: $\bar{x}$ = 3419.92; Max = 7533.61; (3/10) [E]OTA: $\bar{x}$ = 1.53; Max = 3.1; (4/6)		
Gonçalves et al. 2017	January – December 2015	[A]Asia (VN, ID, MM) [E]Europe (DK, AT, NL, DE)	Farm level Feed plant	N_t = 25 [A]n = 21 (20/21) [E]n = 4 (4/4)	Shrimp Fish	AF ZEN DON FUM OTA	[A]AF: $\bar{x}$ = 58; Max = 201 [A]ZEN: $\bar{x}$ = 53; Max = 157 [A]DON: $\bar{x}$ = 29; Max = 63 [A]FUM: $\bar{x}$ = 58; Max= 238 [A]OTA: $\bar{x}$ = - ; Max = 7 [E]AF: not detected [E]ZEN: $\bar{x}$ = -; Max = 6 [E]DON: $\bar{x}$ = -; Max = 20 [F]FUM: n.d. [E]OTA: n.d.	HPLC	------
Marijani et al. 2017	n/a	Kenya Kisumu -> n = 16 Tanzania Ukerewe -> n = 13 Rwanda Kigembe -> n = 10 Uganda Jinja -> n = 13	[FM]Farm [LFP]Local feed plant [IF]Imported feed (from Israel and India) [FI]Feed Ingredients	N_t=52 [FM]n= 14 [LFP]n = 14 [IF]n = 12 [FI]n = 12	Nile tilapia African catfish	3-ADON 15-ADON DON AF DAS AOH FB$_1$ FB$_3$ OTA ROQ-C	[FM]AF = 2.4-126; $\bar{x}$ = 71.0 ± 31.5 [FM]FUM = 33.2-2834.6; $\bar{x}$ = 1136.5 ± 717.9 [FM]DON = 69.1-755.4; $\bar{x}$ = 245.8 ± 190.1 [LFM]AF = <2-28; $\bar{x}$ = 11.6 ± 0.7 [LFM]FUM, DON = <LOD [IF]AF = <2-2.6; $\bar{x}$ = 1.4 ± 0.9 [IF]FUM, DON = <LOD	LC–MS/MS	▪ Farmers who formulate their own feed used: sunflower seed cake, rice bran, cotton seed cake, maize bran and soybean. ▪ Feeds co-contaminated with 12[FM], 4[LFM] and 5[IF] mycotoxins. ▪ NEO, FUX and STERIG were not detected in any of the samples ▪ AF co-occurred with FUM in 13 of 24 feed samples ▪ DON co-occurred with FUM in 2 of 24 feed samples
Gonçalves et al. 2018	January – December 2016	Asia (SAS: IN, ID, MN, TW, TH)	Farm level Feed plant	N_t= 16 [S]n= 4 [F]n= 12	Shrimp[S] Fish[F]	AF ZEN DON FUM OTA NIV 3-AcDON 15-AcDON FUX	[F]AF: $\bar{x}$ = 51.83; Max = 32; (8/12) [F]ZEN: $\bar{x}$ = 75.66; Max = 153; (6/12) [F]DON: $\bar{x}$ = 82.87; Max = 396; (8/12) [F]FUM: $\bar{x}$ = 354.22; Max = 993; (9/12) [F]OTA: $\bar{x}$ = 1.65; Max = 3; (6/12) [S]AF: $\bar{x}$ = 0.43; Max = 24; (4/4) [S]ZEN: $\bar{x}$ = 22.0; Max = 53; (3/4) [S]DON: $\bar{x}$ = 881.66; Max = 2287 (3/4) [S]FUM: $\bar{x}$ = - ; Max = 43; (1/4)	LC-MS/MS	------

			T-2	[S]OTA: $\bar{x}$ = 2.66; Max = 4; (3/4)
			HT-2	
			DAS	
			NEO	

Reference entries are in chronological ordered by sampling date collection or publishing date. Superscript letters give extra information; they are only valid for the same row.

<u>General abbreviations</u>: $\bar{x}$ = average value; $\tilde{x}$ = median value; Max = maximum; HPLC = High-performance liquid chromatography; ELISA = enzyme linked immunosorbent assay; LC–MS/MS = Liquid chromatography-tandem mass spectrometry; TLC = Thin layer chromatography; HPTLC = high performance thin layer chromatography ; LOD = limit of detection; n.d.= not detected

<u>Mycotoxins</u>: AF: aflatoxins (the sum of AFB_1, AFB_2, AFG_1 and AFG_2); AFB_1= aflatoxin B_1; AFB_2= aflatoxin B_2; AFG_1= aflatoxin G_1; AFG_2= aflatoxin G_2; DON = deoxynivalenol; FUM = fumonisins (the sum of FB_1 and FB_2); FB_1= fumonisin B_1; FB_2= fumonisin B_2; OTA= ochratoxin A; ZEA= zearalenone; NIV= Nivalenol; 3-AcDON= 3-Acetyldeoxynivalenol; 15-AcDON= 15-Acetyldeoxynivalenol; FUX= fusarenon X-glucoside; fumonisins; DAS= Diacetoxyscirpenol; NEO= neosolaniol; AOH= alternariol; ROQ-C= roquefortine C; STERIG= sterigmatocystin.

<u>Regions</u>: NAS = northern Asia; SAS = South-East Asia; CN = China; IN = India; TH = Thailand; MN = Myanmar; ID = Indonesia; TW = Taiwan; HR = Croatia; PT = Portugal; DK = Denmark; AT = Austria; NL = the Netherlands; DE = Germany.

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Table 2: Documented aflatoxin carry-over on aquaculture species.

Reference	Species	Tested dosage	Mycotoxin detection level ($\mu g\ kg^{-1}$)	Transfer factor	Method of analysis	Observations
Fish studies						
Suzy *et al.* **2017**	**African sharptooth catfish** *(Clarias Gariepinus)*	10^1, 17^2 and 20^3 $\mu g\ AFB_1\ kg^{-1}$	M^1 = 0.05±0.12 $\mu g\ AFB_1\ kg^{-1}$ M^2 = 0.08±0.10 $\mu g\ AFB_1\ kg^{-1}$ M^3 = 0.08±0.12 $\mu g\ AFB_1\ kg^{-1}$	M^1 = 0.005 M^2 = 0.005 M^3 = 0.004	ELISA	▪ Initial weight: 4±2 g; 3 month study ▪ Chicken droppings were used as ingredient contaminated with 5, 7.2 and 8.2 $\mu g\ AFB_1\ kg^{-1}$ ▪ Catfish fed 10 $\mu g\ AFB_1\ kg^{-1}$ used as control ▪ No differences in haematological parameters
El-Sayed and Khalil, 2009	**European seabass** *(Dicentrarchus labrax)*	[#1]Oral 96h LC_{50} >0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35 and 0.40 mg kg^{-1} [#2]42 day exposure to 10% of oral 96h LC_{50} = 180 $\mu g\ kg^{-1}$	[#2]M = 4.25 ± 0.85 $\mu g\ AFB_1$ kg^{-1}	[#2]M = 0.236	ELISA	▪ Initial weight: 40±2 g ▪ [#1]96h LC_{50} = 0.18 mg/kg bwt ▪ [#2]0.018 mg/kg bwt AFB_1 ▪ [#1,2]Clinical signs: sluggish movement, loss of equilibrium, rapid opercular movement, and hemorrhages of the dorsal skin surface. [#2]Yellowish discoloration, pale discoloration of the gills, liver and kidney. Severe distension of the gall bladder.
Huang *et al.* **2011**	**Gibel carp** *(Carassius gibelio)*	3.2, 11.3, 20.2^1, 55.2^2, 95.8^3, 176.0^4 and 991.5^5 $\mu g\ AFB_1\ kg^{-1}$	L^{1-5} > 5 $\mu g\ AFB_1\ kg^{-1}$ M^5 = 2.35 $\mu g\ AFB_1\ kg^{-1}$	HP^{1-5*}> 0.090 M^5 = 0.0024	ELISA	▪ Initial weight: 10.33±0.19 g ▪ 12 week study ▪ Fish showed strong clearance ability of AFB_1
Raghavan *et al.* **2011**	**Hybrid sturgeon** *(Acipenser ruthenusx A. baeri)*	0, 1, 5, 10, 20, 40^1 and 80^2 μg $AFB_1\ kg^{-1}$	M ≈ 28^1 and 34^2 L = 142.80^1	M^1 = 0.7 M^2 = 0.425	ELISA	▪ Initial weight: 10.53 ± 0.17 g ▪ 35 day study

			and 115.60[2] µg kg^{-1}	$L^1 = 3.57$ $L^2 = 1.15$		▪ Liver hypertrophy and hyperchromasia of nuclei and cytoplasmic vacuoles, presence of inflammatory cells, focal hepatocyte necrosis and extensive biliary hyperplasia.
Lopes et al. 2005	**Jundiá** (*Rhamdia quelen*)	[#1] 41, 90[1] and 204[2] µg AFB$_1$ kg^{-1} [#2] 350[1]; 757[2]; 1,177[3] µg AFB$_1$ kg^{-1}	[#1] M = 1[1] and 6.1[2] µg AFB$_1$ kg^{-1} [#2] M+L=350[1]; 757[2] µg kg^{-1} and 1,177[3] µg AFB$_1$ kg^{-1}	[#1] $M^1 = 0.024$ [#1] $M^2 = 0.030$ [#2] $M+L^1 = 1$ [#2] $M+L^2 = 1$ [#2] $M+L^3 = 1$	HPLC	▪ Initial weight: 3.21[#1] g and 4.73[#2] g ▪ 45[#1] and 35[#2] day studies
Michelin et al. 2016	**Lambari fish** (*Astyanax altiparanae*)	0, 10[1], 20[2] and 50[3] µg AFB$_1$ kg^{-1}	L = 265[2,t] and 243[3,t] µg kg^{-1} M = 19[1,t], 20[2,t] and 50[3,t] µg kg^{-1}	$L^{2,t} = 13.25$ $L^{3,t} = 4.86$ $M^{1,t} = 1.9$ $M^{2,t} = 1$ $M^{3,t} = 1$	HPLC	▪ Initial weight: 3.15 g ▪ 120 day study (sampling at day 30, 60, 90 and 120[t]) ▪ For the first 60 days of exposure, AFs were metabolised by liver and excreted. After 90 days, a lower efficiency in the elimination of AFs
Abdel Rahman et al. 2017	**Nile tilapia** (*Oreochromis niloticus*)	(0 and 200[1] µg AFB$_1$ kg^{-1}) x (FEO + SC)	$L^1 = 5\pm0.5$ µg AFB$_1$ kg^{-1} $M^1 = 3.7\pm0.1$ µg AFB$_1$ kg^{-1}	$L^1 = 0.025$ $M^1 = 0.019$	HPLC	▪ Initial weight: 26.6±0.12 g; 30 day study ▪ Tested fennel essential oil (FEO) and saccharomyces cerevisiae (SC) as mycotoxin management strategy. ▪ AF effects are reported only for 0 and 200[1] µg kg^{-1}
Ayyat et al. 2013	**Nile tilapia** (*Oreochromis niloticus*)	(0, 250[1] µgAFB$_1$ kg feed^{-1}) x OZ, B or C	$M^1 = 78.33$ µg kg^{-1}	$M^1 = 0.313$	HPLC	▪ Initial weight: 7.3 g; 3 week study ▪ Tested ozone (0.5 mg/L/minute; OZ), bentonite (20 g/kg diet; B) and coumarin (5 g/kg diet; C) as detoxifying strategy
Deng et al. 2010	**Nile tilapia** (*Oreochromis niloticus*)	19; 85[0]; 245[1]; 638[3]; 793[3] and 1,641[4] µg kg^{-1}	Y [t1-tf;0-4] $L^{t1} = 10^0, 16^1, 21^2, 24^3$ and 24^4 µg AFB$_1$ kg^{-1} liver $L^{tf} = 30^0, 33^1, 47^2, 44^3$ and 43^4 µg AFB$_1$ kg^{-1} liver	Y [t1-tf;0-4] $L^{t1} = 0.118^0, 0.065^1, 0.033^2, 0.030^3$ and 0.015^4 $L^{tf} = 0.353^0, 0.135^1, 0.074^2, 0.055^3$ and 0.026^4	ELISA	▪ Initial weight: 20 g; ▪ 20[tf] week study (sampling at week 5[t1]) ▪ AF from mouldy peanut meal
Hessein et al. 2014	**Nile tilapia** (*Oreochromis niloticus*)	(0, 250[1] mg^{-1}) x Vit or C	$M^1 =$ mg kg^{-1}	$M^1 = 0.407$	HPLC	▪ Initial weight: 7.3 g; 98 day study ▪ Tested coumarin (5 g/kg diet; C) and vitamin E (50mg kg^{-1} diet; Vit) as detoxifying strategy ▪ No differences on Hb, RBcs, Hct, WBCs, Plat Note: Hessein et al., 2014 reports in his manuscript a residual AF of 107.7 mg kg^{-1}, each seems extremely high, which might be a mistake of units mg kg^{-1}/ µg kg^{-1}
Hussain et al. 2017	**Nile tilapia** (*Oreochromis niloticus*)	(0, 2000[1], 4000[2] mg kg^{-1}) x 0.5% and 1% CB	$M^2 = 0.087\pm1.32$ µg kg^{-1}	$M^2 \sim 0$	HPLC	▪ Initial weight: 4.5±0.4 g; 10 week study ▪ Tested calcium bentonite (CB) clay as detoxifying strategy; ▪ Tested CB significantly improved some parameters (WG, HIS) ▪ CB significantly reduced bioaccumulation of AFB$_1$ residues in muscle tissues.
Mahfouz et al. 2015	**Nile tilapia** (*Oreochromis niloticus*)	20[1] and 100[2] µg AFB$_1$ kg^{-1} feed	L [1,t1] = 5 µg kg^{-1} [1,t2] = 8 µg kg^{-1}	$L^{1,t1} = 0.25$ $L^{1,t2} = 0.4$ $L^{2,t1} = 0.1$	TLC	▪ Initial weight: 35±0.50 g; 6[t1] or 12[t2] week studies ▪ Challenge test with *Aeromonas hydrophila*, IP ▪ Expression of liver *GPx* and *GST* down-regulated[1] ▪ The ability to withstand *A. hydrophila* infection was

Reference	Species	Dose	Detected	Ratio	Method	Notes
			^{2,t1} = 10 µg kg^{-1} ^{2,t2} = 15 µg kg^{-1} M$^{2; t2}$ = 5 µg kg^{-1}	L^{2,t2} = 0.15 M$^{2; t2}$ = 0.05		remarkably lowered
Salem *et al.* 2009	**Nile tilapia** (*Oreochromis niloticus*)	0, 150^{1} µg AFB$_1$ kg^{-1}	M^{1} = 99.48 µg AFB$_1$ kg^{-1}	M^{1} = 0.663	HPLC	▪ Initial weight: 10±3 g; 15 week study ▪ AFB$_1$ was produced through pellet fermentation using *Aspergillus parasiticus* NRRL 2999
Selim *et al.* 2014	**Nile tilapia** (*Oreochromis niloticus*)	(0 and 200^{1} µg kg^{-1}) x HSCAS, SC and EGM	M^{1} ≈ 90 µg kg^{-1}	M^{1} ≈ 0.45	HPLC	▪ Initial weight: 15±2 g; 10 week study ▪ Tested hydrated sodium calcium aluminosilicates (HSCAS; 0.5%), *Saccharomyces cerevisiae* (S.C.; 0.25%) and an esterified glucomannan (EGM; 0.25%) as detoxifying strategy; ▪ AF produced from polished raw rice
Ngethe *et al.* 1993	**Rainbow trout** (*Oncorhynchus mykiss*)	15.6 µg ml^{-1} of AFB$_1$	L$^{1, 2, 4}$ B$^{1, 2, 4}$	n/a	[^{3}H]-AFB$_1$ was measured in a scintillation counter and data expressed in counts per minute (CPM)	▪ Initial weight: 200±20 g; 3 week study (sampling at 6h^1, 1 day^2, 2 days^3 and 6 days^4) ▪ Intravenous injection of 3H-AFB$_1$
Ellis *et al.* 2000	**Rainbow trout** (*Oncorhynchus mykiss*)	20 µg kg^{-1} AFB1 and 20 µg kg^{-1} AFB$_1$ + 2% clay	Detected in: F, K, GI, U, Bi, Ca	n/a	[^{3}H]-AFB$_1$ was measured in a scintillation counter and data expressed in counts per minute (CPM)	▪ Initial weight: 266±12.6 g, 7 day study ▪ 2% sodium bentonite Volclay tested as detoxifying strategy;
Ngethe *et al.* 1992;	**Rainbow trout** (*Oncorhynchus mykiss*)	15.6 µg ml^{-1} of AFB$_1$	Detected in: Bi, L, K, B, AbF, M, Sp and Bl	n/a	[^{3}H]-AFB$_1$ was measured in a scintillation counter and data expressed in counts per minute (CPM)	▪ Initial weight: 100±15 g, 8 day study (sampling at 6h, 1, 2 4 and 8 days) ▪ Intravenous injection and oral dose of ^{3}H-AFB$_1$
Usanno *et al.* 2005	**Red tilapia** (*Oreochromis niloticus x O. mossambicus*)	0, 50, 100, 500, 1,000 and 2,500 µg kg^{-1}	Not detected	n/a	n/a	▪ 8 week trial ▪ No information on fish weight
Husssain *et al.* 1993	**Walleye fish** (*Sander vitreus*)	0, 50 and 100^{1} µg kg^{-1}	Detected in muscle: AFB$_1$^{1} = 5 µg kg^{-1} AFB$_2$^{1} = 10 µg kg^{-1} AFG$_1$^{1} = 15 µg kg^{-1} AFG$_2$^{1} = 20 µg kg^{-1}	AFB$_1$ = 0.5 AFB$_2$ = 0.1 AFG$_1$ = 0.15 AFG$_2$ = 0.2	n/a	▪ 30 day study ▪ No information on fish weight
Shrimp studies						
Boonyaratpalin *et al.* 2001	**Black tiger shrimp** (*Penaeus monodon* Fabricius)	0; 50^{1}; 100^{2}; 500^{3}; 1,000^{4}; 2,500^{5} µg kg^{-1} AFB$_1$	Head and shell / muscle (µg kg^{-1}) ^{1,t1} = 2.6/13.0; ^{1,t2} = 0.5/ 0.4 ^{2,t1} = 3.5/ 14.2; ^{2,t2} = -/ 0.6 ^{3,t1} = 9.1/ 10.6; ^{3,t2} = 6.8/ 0.3 ^{4,t1} = 2.3/8.4; ^{4,t2} = 6.5/0.7 ^{5,t1} = 3.9/7.4; ^{5,t2} = 4.9/0.1	Head and shell / muscle (µg kg^{-1}) ^{1,t1} = 0.052/0.26; ^{1,t2} = 0.01/ 0.008 ^{2,t1} = 0.035/ 0.142; ^{2,t2} = -/ 0.006 ^{3,t1} = 0.0182/ 0.0212; ^{3,t2} = 0.0136/ 0.0006 ^{4,t1} = 0.0023/0.0084;	TLC	▪ Study in adult stage, Initial weight: 1.0-1.2 g; 8 week trial (sampling at 4^{t1} and 6^{t2} weeks)

			[4,t2] = 0.0065/0.0007 [5,t1] = 0.0016/0.0030; [5,t2] = 0.0020/~0			
Bintvihok et al. 2003	**Black tiger shrimp** (Penaeus monodon Fabricius)	5, 10, 20 µg kg^{-1} AFB[1]	not detected	n/a	HPLC	▪ Study in adult stage ▪ 10 day trial ▪ AFB[1] was prepared from mouldy corn
Bautista et al. 1994	**Black tiger shrimp** (Penaeus monodon Fabricius)	25, 50, 75, 100 or 200 µg kg^{-1} AFB[1]	not detected	n/a	HPTLC	▪ Study in adult stage, Initial weight: 17.5±0.6 g ▪ 62 day trial

Reference entries are alphabetically ordered by species common name. Superscript letters give extra information; they are only valid for the same row. Regarding the mycotoxin contamination, when not mentioned it is assumed that a purified form of the respective mycotoxin was used.
General abbreviations: HPLC = High-performance liquid chromatography; ELISA = enzyme linked immunosorbent assay; TLC = Thin layer chromatography; LOD = limit of detection; nd = not detected; n/a = not applicable.
Tissue abbreviations: M = Muscle; L = Liver; HP = hepatopancreas; B = Brain; F = faeces; K = Kidney; GI = Gastro intestinal tract; U = Urine; Bi = Bile; Ca = carcass; AbF = abdominal fat; Sp = spleen and Bl = blood.

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Table 3: Documented ochratoxin carry-over in aquaculture species.

Reference	Species	Tested dosage	Mycotoxin detection level (µg kg^{-1})	Transfer factor	Method of analysis	Observations
Fish studies						
Bernhoft et al. 2017	**Atlantic Salmon** (Salmo salar)	0, 800[1] and 2400[2] µg kg^{-1} OTA	L/M/K/SK (µg kg^{-1}) [1,t1] = 1.86/<LOQ/n.s./n.s. [1,t2] = 1.53/<LOQ/n.s./n.s. [1,t3] = 1.01/<LOQ/0.16/n.s. [2,t1] = 4.81/ <LOQ/n.s./n.s. [2,t2] = 3.27/ <LOQ /n.s./n.s. [2,t3] = 2.61/ <LOQ/1.03/n.s.	L/M/K/SK [1,t1] = 0.0023/<LOQ/n.s./n.s. [1,t2] = 0.0020/<LOQ/n.s./n.s. [1,t3] = 0.0012/<LOQ/~0/n.s. [2,t1] = 0.0020/<LOQ/n.s./n.s. [2,t2] = 0.0013/<LOQ /n.s./n.s. [2,t3] = 0.0011/ <LOQ/~0/n.s.	HPLC	▪ Initial weight: 58 g ▪ Administration of 14C-OTA A and autoradiography ▪ Sampling at 3[t1], 6[t2] and 8[t3] weeks
Fuchs et al. 1986	**Rainbow trout** (Salmo gairdneri)	IV injection of 0.160 µg kg^{-1}	Blood = Detected [t1-t4] Pronephros = Detected [t1-t4] Opisthonephros = Detected [t1-t4] Urine = Detected [t1-t4] Pseudobranch = Detected [t1-t4] Gills = Detected [t1-t4] Liver = Detected [t1-t4] Bile = Detected [t1-t4] Ventricle wall = Detected [t1-t4] l'yloric appendices = (contents) = Detected [t1-t4] Large intestine (contents) = Detected [t1-t4]	n/a	LC fluorometer	▪ Initial weight: 50 g, 8 week study ▪ Sampling at 5 min[t1], 6[t2] and 8[t3] weeks ▪ Fish each was sacrificed at 5[t1] min, 1[t2] hr, 24[t3] hrs and 8[t4] days after injection.

			Splccn ("patches") = Detected [t1-t4]		
			Muscle (close to the myomeres) = Detected [t1-t2]		
			Spinal cord = Detected [t1-t3]		
			Fins = Detected [t1-t4]		
			Skin = Detected [t1-t4]		
			Muscles = Detected [t1-t2]		

Shrimp studies

Supamattaya *et al.* **2005**	**Black tiger shrimp black** (*Penaeus monodon Fabricius*)	100; 200 and 1,000 µg kg^{-1}	Not detected	n/a	HPLC	• Initial weight: 2 g; 8 week study • No differences on THC or Ca^{2+} levels • No differences in tissues: G, AG, HP, HT, * LOD given in the manuscript (44,000 µg kg^{-1}) seems to be very high; there is a chance of an error in the units

Reference entries are alphabetically ordered by species common name. Superscript letters give extra information; they are only valid for the same row. Regarding mycotoxin contamination, when not mentioned, it is assumed that a purified form of the respective mycotoxin was used.

<u>General abbreviations</u>: HPLC = High-performance liquid chromatography; LC = liquid chromatography; n/a = not applicable; n.s. not sampled

<u>Tissue abbreviations</u>: M = Muscle; L = Liver; K = Kidney; SK = skin.

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Table 4: Documented deoxynivalenol and/or fumonisin carry-over in aquaculture species.

Reference	Species	Tested dosage	Mycotoxin detection level (µg kg^{-1})	Transfer factor	Method of analysis	Observations
Fish studies						
Bernhoft *et al.* **2017**	**Atlantic salmon** (*Salmo salar*)	0; 2000[1] and 6000[2] µg kg^{-1} DON	L/M/K/SK (µg kg^{-1}) [1,t1] = 12.2/5.6/n.s./n.s. [1,t2] = 12.8/8.5/n.s./n.s. [1,t3] = 18.1/6.0/12.3/n.s. [2,t1] = 9.6/10.3/n.s./n.s. [2,t2] = 20.2/17.3/n.s./n.s. [2,t3] = 28.6/18.6/16.8/20.8	L/M/K/SK [1,t1] = 0.0061/0.0028/n.s./n.s. [1,t2] = 0.0064/0.0042/n.s./n.s. [1,t3] = 0.0091/0.003/0.0061/n.s. [2,t1] = 0.0016/0.0017/n.s./n.s. [2,t2] = 0.0034/0.0029/n.s./n.s. [2,t3] = 0.0048/0.0031/0.0028/0.0035	HPLC	• Initial weight: 58 g, 8 week study; • Sampling at 3[t1], 6[t2] and 8[t3] weeks

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Nácher-Mestre *et al.* 2015	**Atlantic salmon** (*Salmo salar*)	Diet 1 = 22.4 DON + 148 FUM Diet 2 = 19.4 DON + 754 FUM Diet 3 = 23.1 DON + 112 FUM	Not detected	n/a	LC–ESI–MS/MS	▪ 6 month trial ▪ Initial body weight of 228±5 g ▪ Minor amounts of T-2 found and 15-AcDON and OTA detected
Pietsch *et al.* 2014	**Common carp** (*Cyprinus carpio*)	352[1], 619[2] and 953[3] µg kg^{-1} DON	Muscle samples (µg kg^{-1}) [1] = 0.6; [1, RP] = 1.4 [2] = 1.3; [2, RP] = 0.7 [3] = 1.2; [3, RP] = 0.0	Muscle samples [1] = 0.0017; [1, RP] = 0.0040 [2] = 0.0021; [1, RP] = 0.0011 [3] = 0.0013; [1, RP] = 0	HPLC	▪ Raised from eggs (average initial weight 36 g), 4 week study ▪ Additional 2 weeks of feeding uncontaminated diet – recovery period[RP]
Nácher-Mestre *et al.* 2015	**Gilthead sea bream** (Sparus aurata)	Diet 1 = 79.2 DON + 8.1 15-AcDON Diet 2 = 53.5 DON + 13.6 15-AcDON +6.4 FUM	Not detected	n/a	LC–ESI–MS/MS	▪ 8 month trial ▪ Initial body weight of 15 g up to 296 – 320 g
Huang et al. 2018	**Grass carp** (*Ctenopharyngodon idella*)	27; 318[1]; 636[2]; 922[3]; 1,243[4] and 1,515[5] µg kg^{-1} DON	PI= 16.46[4]; 17.64[5] µg kg^{-1} tissue MI= 15.90[3]; 18.54[4]; 20.34[5] µg kg^{-1} tissue DI= 18.91[3]; 24.40[4]; 28.82[5] µg kg^{-1} tissue	PI= 0.013[4]; 0.012[5] MI= 0.017[3]; 0.015[4]; 0.013[5] DI= 0.021[3]; 0.020[4]; 0.019[5]	HPLC	● Initial weight: 12.17 ± 0.01 g; 60 days trial ● Malformations: missing of pelvic fin[2]; caudal fin deformity[3]; operculum ● "the safe dose of DON for grass carp were all estimated to be 318 µg/kg diet"; Huang *et al.* 2018
Shrimp studies						
Supamattaya *et al.* 2005	**Black tiger shrimp black** (*Penaeus monodon Fabricius*)	500; 1,000 and 2,000 µg kg^{-1} DON	Not detected	n/a	HPLC	▪ Initial weight: 2 g; 8 week study ▪ No differences on THC or Ca^{2+} levels ▪ No differences in tissues: G, AG, HP, HT, * LOD given in the manuscript (50,000 µg kg^{-1}) seems to be very high; there is a chance of an error in the units
Trigo-Stockli *et al.* 2000	**Pacific white shrimp** (*Litopenaeus vannamei*)	0, 200, 500 and 1,000 µg kg^{-1} DON	Not detected	n/a	HPLC	▪ Initial weight: 1.7±0.05 g, 16 week study (sampling at 4, 8, 12 and 16 weeks) ▪ Naturally contaminated hard red winter wheat
Deng et al. 2017	**Pacific white shrimp** (*Litopenaeus vannamei*)	0; 500[1]; 1,200[2]; 2,400[3]; 4,800[4]; 12,200[5] µg kg^{-1} T-2	[HP]m= 17.52±2.87[4] ŋg g^{-1} [HP]m= 48.61±3.13[5] ŋg g^{-1}	n/a	TSQ	● Initial weight: 8.5±0.5 g; 20 days study ● Dietary concentrations correspond to $^1/_{50}$, $^1/_{20}$, $^1/_{10}$, $^1/_5$ and $^1/_2$ (Wang et al. 2015).

Reference entries are alphabetically ordered by species common name. Superscript letters give extra information; they are only valid for the same row. Regarding mycotoxin contamination, when not mentioned, it is assumed that a purified form of the respective mycotoxin was used.

<u>General abbreviations</u>: HPLC = High-performance liquid chromatography; LC–ESI–MS/MS = liquid chromatography-electrospray ionization-tandem mass spectrometry; TSQ= Quantum Access tandem mass spectrometer n/a = not applicable; n.s. not sampled

<u>Tissue abbreviations</u>: M = Muscle; L = Liver; K = Kidney; SK = skin.

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Table 5: Documented zearalenone carry-over in aquaculture species.

Reference	Species	Tested dosage	Mycotoxin detection level (µg kg^{-1})	Transfer factor	Method of analysis	Observations
Fish studies						
Pietsch et al. 2015	**Common Carp** (*Cyprinus carpio* L.)	0; 332[1]; 621[2] and 797[3] µg kg^{-1}	Muscle ZEN[1] = 0.13±0.03 µg kg^{-1} ZEN[2] = 0.22±0.18 µg kg^{-1} ZEN[3] = 0.15± 0.07 µg kg^{-1} α-ZEN[1] = 0.11±0.03 µg kg^{-1} α-ZEN[2] = 0.16±0.011 µg kg^{-1} α-ZEN[3] = 0.05± 0.07 µg kg^{-1} ZEN[1, RP] = 0.03±0.03 µg kg^{-1} ZEN[2, RP] = 0.03±0.02 µg kg^{-1} ZEN[3, RP] = 0.03±0.03 µg kg^{-1}	Muscle ZEN[1] ~ 0 ZEN[2] ~ 0 ZEN[3] ~ 0 α-ZEN[1] ~ 0 α-ZEN[2] ~ 0 α-ZEN[3] ~ 0 ZEN[1, RP] ~ 0 ZEN[2, RP] ~ 0 ZEN[3, RP] ~ 0	HPLC	● Raised from egg with 12-16 cm in length ● 4 week study ● α-ZEN were not detectable after recovery period (2 weeks) and ZEN was detected at 0.03 µg kg^{-1} dry weight for all treatments
Woźny et al. 2015	**Rainbow trout** (*Oncorhynchus mykiss*)	1,810 µg kg^{-1}	Intestines ZEN = 732.2 µg kg^{-1} α-ZEN = 10.7 µg kg^{-1} L = residual ZEN and α-ZEN in all sampled fish	Intestines ZEN = 0.40 α-ZEN = 0.0059	HPLC	● Initial weight: 250 g, all females; 71 day study ● Some animals were identified as males ● ZEN was detected (<5.0 µg kg^{-1}) in all female ovaries
Woźny et al. 2017	**Rainbow trout** (*Oncorhynchus mykiss*)	1 mg kg^{-1} of body mass	ZEN/α-ZEN/β-ZEN (µg kg^{-1}) I[48h] = ~1500/~600/- I[96h] = ~1500/~900/- L[48h] = ~700/~100/~500 L[96h] = <200/<20/~0 O[48h] = 321/~100/- O[96h] = <100/<100/- Oo[48h] = ~25/~10/- Oo[96h] = <5/<5/- P[48h] = ~10/~5/- P[96h] = ~0/~0/- M[48h] = ~5/~5/- M[96h] = ~3/~3/-	ZEN/α-ZEN/β-ZEN (µg kg^{-1}) I[48h] = 1.5/ 0.6/- I[96h] = 1.5/ 0.9/- L[48h] = 0.7/ 0.1/ 0.5 L[96h] = <0.2/<0.02/~0 O[48h] = 0.321/ 0.1/- O[96h] = <0.1/<0.1/- Oo[48h] = ~0.025/~0.01/- Oo[96h] = <0.005/<0.005/- P[48h] = ~0.01/~0.005/- P[96h] = ~0/~0/- M[48h] = ~0.005/~0.005/- M[96h] = ~0.003/~0.003/-	HPLC-FLD	● Initial weight: 1274±162 g, all mature females ● Objective was to study the ZEN carry-over to eggs ● Administration on ZEN – oral (bolus) ● Sampling periods: 2, 6, 12, 24, 48, 72, 96h ● Verified the presence of ZEN and α-ZEN in commercial fish roe ● "Contamination of fish roe with zearalenone residuals is unlikely to pose a health risk to consumers, but their potential to transfer to somatic cells in fish ovaries may be of concern for aquaculture", Woźny et al. 2017

Shrimp - no studies

Reference entries are alphabetically ordered by species common name. Superscript letters give extra information; they are only valid for the same row. Regarding mycotoxin contamination, when not mentioned, it is assumed that a purified form of the respective mycotoxin was used.

General abbreviations: HPLC = High-performance liquid chromatography; HPLC-FLD = High-performance liquid chromatography with fluorescence detection

Tissue abbreviations: I = Intestines; O = Ovaries; Oo = Oocytes; P = Plasma, M = Muscle

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Table 6: Documented mycotoxin occurrence in commercial aquaculture foods.

Reference	Sampling Country (region)	# samples / Species	Sample origin	Target mycotoxin analysed in tissue	Tissue sampled	Mycotoxin detection level (µg kg^{-1})	Method of analysis	Observations
Tolosa et al. 2013	Spain (Valencia)	N_t = 19 n = 9 [SB]Seabass[AQ] n = 5 [GSB]Seabream[AQ] n = 3 (mackerel, hake, cod)[WF] n = 1 [T]Tilapia[AQ] n = 1 [P]Panga[AQ]	Aquaculture[AQ] ▪ Seabass Spain (Cartagena, Murcia) Greece (Argolis) ▪ Seabream Spain and Greece (Argolis); ▪ Tilapia China ▪ Pangasius Vietnam Wild fisheries[WF] ▪ Hake Southeast Atlantic ▪ Cod and Mackerel Northwest Atlantic	BEA ENA ENA1 ENB ENB1	Muscle	ENA1[SB] = 1.70±0.07 to 6.91±0.12; 4/9 n.d. ENA1[GSB] = 2.48±0.07 to 7.45±0.12; 2/5 n.d. ENA1 = 1.51±0.07[T]; n.d.[P] ENB[SB] = 3.60±0.08 to 44.65±0.12; 1/9 n.d. ENB[GSB] = 1.30±0.08 to 21.63±0.11; 1/5 n.d. ENB = 5.35±0.07[T]; 1.26±0.06[P] ENB1[SB] = 1.44±0.09 to 31.51±0.11; 2/9 n.d. ENB1[GSB] = 7.13±0.1 to 18.95±0.12; 2/5 n.d. ENB1 = 2.20±0.07[T]; n.d.[P] ENA1 / ENB / ENB1[WF] = nd	LC–MS/MS	▪ ENA and BEA were not detected in samples analysed ▪ Seabass (*Dicentrarchus labrax*) ▪ Seabream (*Sparus aurata*) ▪ Aquaculture[AQ] ▪ Wild fisheries[WF]
Woźny et al. 2013	Poland (North-eastern region)	N_t = 9 3 samples from 3 different farms[(F1 to F3)]	Poland (North-eastern region)	ZEN	Intestine Liver Ovary Muscle	Intestine = n.d. [F1]; <2.0 [F2]; <2.0 [F3] Liver = n.d. [F1]; <2.0 [F2]; nd [F3] Ovary = <2.0 [F1]; =7.1±3.2 [F2]; <2.0 [F3] Muscle = n.d. [F1 to F3] Water = n.d. [F1 to F3]	HPLC	
Woźny et al. 2017	Poland 2013[T1], 2014[T2], 2015[T3]	n = 35 (acquired from hatcheries)[AQH] n = 6 (from supermarket)[S]	Norway Poland	ZEN, α-ZEL, β-ZEL	Ovary[Ov] Oocytes[Oo] Salted roe[Sr]	ZEN, α-ZEL, β-ZEL[Ov] = Detected in 4/4 samples [T2; Om, Sf] and in 1/6 samples [T3; Om, Ss] ZEN, α-ZEL, β-ZEL[Oo] = Detected in 5/13 samples [T2; Ao, Cl, Ci, Hm, Om, Sf, Sg]; in 5/6 samples [T2; Cl, Ok, Om, Sf] and in 2/6 samples [T3; Ok, Om, Ss] ZEN, α-ZEL, β-ZEL[Sr] = Detected in 0/1 [T1]; in 2/3 samples [T3; Ok, Om] and in 2/2 samples [T3; Ok, Om] [#1]α-ZEL[Ov] = 14.5 [T2; Om] [#1]α-ZEL[Ov] = 12.6 [T2; Sf] All mycotoxin levels detected below LOD (ZEN, a-ZEL, and ß-ZEL were 5.0, 3.0, and 12.0 ug kg-1) except [#1]	HPLC-FLD	Species sampled: *Acipenser oxyrinchus*[Ao] *Coregon lavaretus*[Cl] *Ctenopharyngodon idella*[Ci] *Hypophthalmichthys molitrix*[Hm] *Oncorhynchus mykiss*[Om] *Salvelinus fontinalis*[Sf] *Silurus glanis*[Sg] *Oncorhynchus keta*[Ok] *Salmo salar*[Ss]

Reference entries are alphabetically ordered by publication first author. Superscript letters give extra information; they are only valid for the same row.

General abbreviations: HPLC = High-performance liquid chromatography; HPCL-FLD = high-performance liquid chromatography: fluorescence detection; LC–MS/MS = Liquid chromatography-tandem mass spectrometry; n.d. = not detected

Mycotoxins: BEA = beauvericin; ENA = enniatins; ENA1 = enniatin A1; ENA2 = enniatin A2; ENB = enniatin B; ENB1 = enniatin B1; ZEN = zeralenone; α-ZEL = alpha-Zearalenol; β-ZEL = beta-Zearalenol.

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