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Keywords:	mycotoxins occurrence, carry-over effects, fish, Shrimp, aquafeeds, transfer factor

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

Mycotoxin abbreviations:

AFs: aflatoxins; meaning 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

ENNs: enniatins

FUM: fumonisins; meaning the sum of FB_1 and FB_2

FB_1: fumonisin B_1

FB_2: fumonisin B_2

OTA: ochratoxin A

ZEN: zearalenone

α-ZEL: alpha-Zearalenol

β-ZEL: beta-Zearalenol

Other abbreviations:

TF – Transfer factor

DN – Denmark

AT – Austria

NL – the Netherlands

DE – Germany

VN – Vietnam

ID – Indonesia

MM - Myanmar

INTRODUCTION

Mycotoxins are secondary metabolites produced by some molds (Hussein and Brasel, 2001). These can be produced on agricultural commodities pre- and/or post-harvest including directly in finished feeds. Mycotoxins are responsible for significant economic losses due to the spoilage of agricultural products (CAST, 2003; Shane and Eaton, 1994; Vasanthi and Bhat, 1998). Furthermore, mycotoxins can cause diseases problems when consumed by humans and livestock, causing significant problems worldwide (Zain, 2011). Despite being identified as categorically undesirable for most aquaculture species, their occurrence, at least in field conditions, is not completely preventable even when using good manufacturing practices (FAO 1979). The awareness of mycotoxin-related issues in the aquaculture industry has been increasing, accentuated by the increased inclusion levels of plant meals in aquafeeds (Tacon *et al.* 2011). Traditionally, the use of minor amounts of plant feed stuffs led to an accepted perception that mycotoxins were not a relevant issue in aquaculture and that the majority of mycotoxin issues would stemmed only due to poor storage conditions. *Aspergillus* spp. and *Penicillium* spp. can grow on feed stored in poor conditions, ultimately leading to the production of aflatoxin (AF) and ochratoxin A (OTA). This would seem to be particularly the case in countries where climate conditions are favourable to the growth of *Aspergillus* spp. and *Penicillium* spp. fungi. However, optimal storage conditions should prevent the contamination of raw materials and finished feeds from AF or OTA. However, some plant commodities such as cottonseed and peanut meals commonly present detectable levels of AF and/or OTA (Gonçalves *et al.* 2017), even when stored using appropriate conditions.

With the increased use of plant meals in aquafeeds, other mycotoxins besides AF and OTA have been reported in finished feeds, as mycotoxins are reasonably stable to processing conditions (Cheli *et al.* 2013). *Fusarium* mycotoxins (Type B and A, trichothecenes and fumonisins) are, contrary to AF and OTA, mainly produced at pre-harvest stage. The production of these mycotoxins by *Fusarium* spp. seems to be highly influenced by environmental conditions, so an increase in occurrence is expected due to climate change (Miraglia *et al.* 2009; Paterson and Lima, 2010; Paterson and Lima, 2011). This contamination may potentially cause harm to the fish and shrimps, dependent upon mycotoxin concentration and co-occurrence, consequently resulting in significant economic losses, directly (e.g., mortality or decreases in performance), or indirectly (e.g. higher susceptibility to diseases). However, one of the biggest barriers to quantify the impact of mycotoxin

contamination in the aquaculture industry is the apparent lack of clinical signs or biomarkers in aquatic species for mycotoxin exposure, especially compared to terrestrial livestock. While several reports describe broad and non-specific clinical signs for the most common mycotoxins (see review from Anater *et al.* (2016)), these lack specificity and could be attributed to a number of pathologies or challenges such as the presence of anti-nutrition factors or lectins in the diet (Hart *et al.* 2010). The case of aflatoxicosis, (yellowing of the body surface, (Deng *et al.* 2010) and ingestion of fumonisins (FUM; alteration of the sphinganine to sphingosine ratio, (Tuan *et al.* 2003)) are two notable exceptions. Also, Gonçalves *et al*. (2018b) described DON-3-sulfate as a potential biomarker of deoxynivalenol (DON) exposure in rainbow trout (*Oncorhynchus mykiss*).

Carry-over denotes the conveyance of undesired compounds from contaminated feed into food of animal origin. The potential of carry-over of several mycotoxins in terrestrial animals such as poultry, swine and cows issue was highlighted by the European Food Safety Authorities (EFSA) and FAO (Domenico Caruso *et al.* 2013; EFSA, 2004b FAO, 2001)). However, no guidelines are available regarding carry-over in farmed fish and shrimp species.

Therefore, the present review aims to compare the mycotoxin occurrence and co-occurrence in aquaculture finished feeds with the potential risk of mycotoxin carry-over in aquaculture seafood products across main aquaculture produced species. Furthermore, we aim to critically compare carry-over obtain in aquaculture species to the ones obtained for livestock species. With this review, we intend to raise awareness to the scientific community, the regulatory authorities and the aquaculture industry to the possible need for specific aquaculture mycotoxin maximum concentration levels for both aquaculture feeds and foods. Furthermore, authors aware for particular cases in aquaculture sector, where edible tissues may change in different regions, therefore increasing the risk of mycotoxicosis.

OCCURRENCE OF MYCOTOXINS IN AQUAFEEDS

The high cost and limited availability of fishmeal has led the aquaculture industry to gradually increase the levels of alternative protein sources as a substitute for fishmeal in their feeds (Davis and Sookying, 2009). Overall, a wide range of products, e.g. animal by-products, fishery by-products, insect meals, macro-algae meals or single-cell protein, have been explored as alternatives to fishmeal. However, for several reasons (e.g., production scalability, market availability, batch uniformity or price competitiveness) plant-based meals

remain the most widely used alternative protein source. When considering plant-based meals for aquafeeds, it is commonly agreed that one of the negative aspects is the presence of anti-nutrients (e.g. cyanogens, saponins, tannins etc.) which are detrimental to fish and shrimp (Krogdahl *et al*. 2010). Conversely, the negative impact of mycotoxins is often overlooked. The disbelief in the negative effects of mycotoxins on aquatic species might be related to the lack of observable clinical signs in aquatic species directly related to mycotoxin ingestion compared to terrestrial livestock species where the effects are more pronounced. However, the awareness of mycotoxin-related issues in the aquaculture industry has grown in recent years as feed manufacturers and producers have recognised the importance of mycotoxins and their potential to impact production, final product quality (García-Morales *et al.* 2013) and safety for consumers (Michelin *et al.* 2017). The evolution of the analytical platforms used to detect mycotoxins and the easier access to analytical labs or simple ELISA strip tests kits for *in situ* testing, has also increased the awareness of mycotoxins to feed millers and farmers.

During the revision of the peer-reviewed literature on the occurrence of mycotoxins in aquafeeds, summarized in this review, a pattern of the target mycotoxins analysed in feed samples emerged. In samples analysed before 2012, the main mycotoxins analysed were AFs (AFB_1, AFB_2, AFG_1, AFG_2; in most of the cases only AFB_1; see Table 1) and in some cases zeralenone (ZEN) and OTA (Fegan and Spring 2007) (with the exception of (Martins *et al.* 2008) and, possibly based on previous data reported on terrestrial livestock feed samples. After 2012, other mycotoxins were beginning to be reported besides AF's (Table 1). These studies have either targeted the analysis of specific mycotoxins due to the inclusion of certain plant meals (e.g., (Pietsch *et al.* 2013; Woźny *et al.* 2013) or explored a broad mycotoxin occurrence (Gonçalves *et al.* 2018a; Gonçalves *et al.* 2018; Gonçalves *et al.* 2017; Nácher-Mestre *et al.* 2015). This different pattern in the target mycotoxin analysed in feed might be a reflection of increasing awareness of mycotoxins in aquaculture, but also as a result of the easier access to mycotoxin analytical methods.

Aquafeed studies with samples preceding 2012

The oldest documented survey of mycotoxin occurrence in aquaculture finished feed was by Bautista *et al.* (1994). In this study, a total of 62 samples collected in the Philippines between August 1990 to February 1991 from black tiger shrimp (*Penaeus monodon*) feed, sourced

from feed mills and at farm level were analysis (Table 1). The authors observed that only two of the 62 samples were free from AFs, 36 samples were contaminated with AFs at levels between 10 and 20 µg kg^{-1}, 21 samples contained AFs at levels between 30 and 40 µg kg^{-1} and two samples had AFs levels of 60 and 120 µg kg^{-1}. The second study was from Bintvihok *et al*. (2003) which analysed samples collected in the eastern and southern regions of Thailand (1997 to 1998) and by Altuğ and Berklevik (2001) with samples collected in Turkey from 1998 to 2000 (Table 1). Bintvihok *et al*. (2003) analysed 150 samples of commercial shrimp feed (formulated for *Penaeus monodon*) composed mainly of fishmeal, soybean and corn. Samples were collected directly from farms in ten different provinces during the summer months (March to June 1997), the rainy season (July to October 1997) and the winter (November to February 1998) and analysed for AFB_1, AFB_2, AFG_1 and AFG_2. Bintvihok *et al*. (2003) observed that feed was more frequently contaminated in the eastern region (43 contaminated out of 75 collected samples) compared to the southern region (14 contaminated out of 75 collected samples). Contamination also occurred more frequently during rainy season (29 contaminated out of 50 collected samples) followed by winter (20 contaminated in 50 collected samples). AFB_1 was the most prevalent mycotoxin found in samples, although at relatively low concentrations (< 1 µg kg^{-1}; Table 1). However, the study lacked information regarding levels of inclusion of the plant ingredients as well as storage time and conditions prior to analysis, which does not allow drawing further conclusions regarding the origin of the AF contamination (i.e., from raw materials or contamination during storage). Soybean and corn are not typically contaminated with AFs, at least in the field, as these plant commodities are more likely contaminated with DON, FUM and ZEN (Gonçalves *et al.* 2018a). Therefore, AF contamination in finished feeds could reflect inadequate storage conditions of raw materials or feeds. Reporting inclusion levels of plant ingredients would be very useful. Importantly, Altuğ and Berklevik (2001) analysed 170 fish finished feed samples for the presence of AFB_1 in Turkey between 1998 and 2000. Samples were collected at farm level, feed mills or imported feeds. In this study, AFB_1 was found below detection limits in 43 samples (25.2% of samples), in 20 samples (11.7% of samples) AFB_1 levels were above 20 µg kg^{-1} and in 85 samples (50% of samples) AFB_1 ranged between 21.2 to 42.4 µg kg^{-1}. Authors from this study concluded that levels of AFB_1 were higher in samples taken from farms compared to feed mill or imported feed samples.

Fegan and Spring (2007) reported, to our knowledge, the first and most complete mycotoxin occurrence survey on fish and shrimp feeds before 2012. Samples were collected in India and Thailand and analysed for the presence of AFs, T-2, ZEN and OTA. No information is available on the period of sampling, region area or sample origin (feed mill or farm). Nonetheless, the information reported shows a different contamination pattern between fish and shrimp feeds and also shows co-occurrence of mycotoxins. Out of the nine fish feed samples analysed from Thailand, all samples were contamination predominantly by ZEN, at 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 (2.32 to 7.74 µg kg^{-1}) . Also in Thailand, shrimp feed samples (n=7) were contaminated with ZEN and OTA while no data on AFs was available (Table 1). Shrimp feed samples (n=10) collected from India were mostly contaminated with AFs, ranging between 40 and 90 µg kg^{-1}. However, it is important to mention that levels of sensitivity are mycotoxin-specific and therefore although OTA reported levels were in general lower than ZEN, aquatic species are more sensitive to OTA (see Gonçalves *et al.* 2018 for sensitivity levels in aquatic species). In their study, Fegan and Spring (2007) also reported mycotoxin occurrence in the raw materials used to formulate aquafeeds. While the objective of the present review is only to report mycotoxin occurrence in finished feed, it is inevitable and fundamental to highlight the occurrence of mycotoxins (T-2 and ZEN and OTA) in marine ingredients (fishmeal from China, Myanmar, Thailand; fish and shrimp meal from Thailand) which will be further discussed in next sections.

An exception to the almost exclusive AF analysis in finished feeds prior to 2012, are the results presented by Martins *et al*. (2008), who analysed 20 samples of fish feed sourced from Portugal for the presence of AFB_1, OTA, DON, ZEN and fumonisin B1 (FB_1). In this study, no detectable levels of the target mycotoxins were obtained.

In the remaining studies shown in Table 1, in which samples were collected in or before 2012 (Alinezhad *et al.* 2011; Almeida *et al.* 2011; Gonçalves-Nunes *et al.* 2015), the target mycotoxin analysed in feed was always AFB_1. Almeida *et al*. (2011), did not detect AFB_1 in the 87 samples of seabass feed collected in Portugal. Interestingly, 35 of the 87 samples analysed were contaminated with *Aspergillus* spp., which highlights that the presence of fungi does not necessarily mean the presence of the toxin and vice-versa. Alinezhad *et al.* (2011), detected levels high concentrations of AFB_1 in fishmeal (average = 67.35 µg kg^{-1}). In

Brasil, Gonçalves-Nunes e*t al*. (2015), reported the presence of AFB_1 ranging from 1.6 to 9.8 µg kg^{-1} in samples collected directly at the feed plant.

Aquafeed samples after 2012

From 2012 onwards, the number of peer-reviewed publications and technical articles (not covered in this review) related to the presence of mycotoxins (including not only AFBs) in aquaculture feeds increased considerably. In 2013, Woźny *et al*. (2013) analysed the presence of ZEN in trout feed collected from three farms in November. One of the farms had no detected levels of ZEN while the other two farms had 81.8 ± 25.8 and 10.3 ± 0.9 µg kg^{-1} of ZEN in their feed respectively. The same study also explored the carry-over of ZEN from feed by analysing several rainbow trout (*Oncorhynchus mykiss*) organs for ZEN presence, results that are further explored in next section. Pietsch *et al*. (2013), unveiled the presence of DON (236.18 µg kg^{-1}) and ZEN (63.82 µg kg^{-1}) in common carp (*Cyprinus carpio*) feeds in samples from central Europe. Still in Europe, Nácher-Mestre *et al*. (2015), investigated the occurrence of mycotoxins in Atlantic salmon (*Salmo salar*) and gilthead sea bream (*Sparus aurata*) feeds, with respectively, high and low inclusion of plant meals. From the 18 mycotoxins analysed, the most representative mycotoxins found were FUM and DON. In Atlantic salmon, from the three types of feeds analysed, levels of DON were 22.4, 19.4 and 23.1 µg kg^{-1} and 148, 754 and 112 µg kg^{-1} of FUM respectively. For gilthead sea bream, two 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 one of the samples. In Argentina, Greco *et al*. (2015) also analysed salmonids feeds. In this study, 28 samples of rainbow trout (*Oncorhynchus mykiss*) feed were sampled at the farms, ranging throughout the feed portfolio for different development stages (starter feed (13 samples); grower feed (13 samples); 4 pigmented and 9 unpigmented feed and finisher feed (2 pigmented samples). The authors observed median values of: AFs = 2.82; OTA = 5.26; T-2 = 70.08; DON = 230 and ZEN = 87.97 µg kg^{-1}. It was also highlighted that, there was a co-occurrence of at least two out of six mycotoxins in 93% (26/28) of the analysed samples. Gonçalves *et al*. (2018a; 2018; 2017) focused on unveiling the mycotoxin occurrence in plant meals (not reported here) and aquaculture finished feeds in Europe and Southeast Asia. In 2014, from January to December, 41 fish and shrimp feed samples were collected from Europe (n = 6 to 10; Croatia and Portugal) and SE Asia (n = 31; Singapore, India, Thailand and

Myanmar). Samples were analysed for AFs, ZEN, DON, FUM and OTA (Table 1). Interestingly, a higher occurrence of FUM was found in European samples (average 3419.92 and maximum 7533.61 µg kg^{-1}) compared to SE Asia. The remaining mycotoxins showed similar occurrence average and maximum levels for Europe and SE Asia, with mycotoxins being detected in all analysed samples. In this mycotoxin survey (Gonçalves *et al.* 2018), it was reported that in Europe, 50% of the samples had more than one mycotoxin per sample, and in Asia, 84% of the samples were contaminated with more than one mycotoxin per feed sample.

In 2015, analysing the same mycotoxins as in the previous study, Gonçalves *et al*. (2017) sourced 25 samples of fish and shrimp feeds in Europe (n = 4; Denmark, Austria, Netherlands and Germany) and SE Asia (n = 21; Vietnam, Indonesia, Myanmar). Contrary to samples collected in 2014, the European samples analysed in 2015 showed relatively low mycotoxin contamination, with only DON contamination reaching values up to 20 µg kg^{-1}. In SE Asian samples, contamination was also generally lower when compared to the previous year, with only AFs showing similar contamination levels to 2014 (average contamination of 58 µg kg^{-1} and maximum of 201 µg kg^{-1}). However, the co-occurrence risk increased in both regions.

From January to December 2016, Gonçalves *et al*. (2018a) sampled four shrimp feeds from India and 12 fish feeds from Indonesia, Myanmar, Taiwan and Thailand. Interestingly, the fish and shrimp feeds showed a relatively different mycotoxin contamination pattern, possibly due to the type of raw materials used to manufacture these diets. Fish feed samples showed lower contamination (Table 1), when compared with shrimp feeds. However, a higher number of co-occurring mycotoxins were observed in fish feeds. Shrimp feeds showed a relatively high contamination of DON, with an average contamination level of 881.66 and maximum of 2287 µg kg^{-1}.

Mycotoxins also represent a big challenge to the increasingly successful aquaculture sector on the African continent. Marijani *et al*. (2017), analysed mycotoxin occurrence in Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) feeds, gathering 16 samples from Kisumu, Kenya, 13 samples from Ukerewe, Tanzania, 10 samples from Kigembe, Rwanda and 13 samples from Jinja, Uganda. Samples were collected from farms (farm-made feeds; n = 14), local feed millers (n = 14) or imported feeds from Israel and India (n = 12). From the 52 samples analysed, Marijani *et al*. (2017) observed that farm-made feeds were highly contaminated with AF, FUM and DON (Table 1). On the other hand, feed samples from local feed millers, as well as the imported feed samples, had only minor contamination of AF.

Discussion on the occurrence of mycotoxins in aquafeeds

From the documented peer-reviewed literature, it is possible to observe a growing interest in the occurrence of mycotoxins in aquatic feeds. It is also observable that there is a shift regarding the target mycotoxins analysed in feeds. Most of the earlier studies evaluating mycotoxins in aquafeeds (Bintvihok *et al.*, 2003, Altuğ and Berklevik, 2001) mainly focused on aflatoxin occurrence and only in recent years, other mycotoxins were analysed. This research pattern, i.e., high focus on AFs and only later on other mycotoxins, can also be observed in the peer-reviewed literature studying the impact of mycotoxins in aquatic animal health and performance (Gonçalves *et al.* 2018). The increasing interest in mycotoxins in aquafeeds, and particularly the interest in other mycotoxins besides AFs, is certainly related to the increasing inclusion levels of plant meals in aquafeeds, as well as, the awareness of mycotoxins conveyed from these plant meals to aquafeeds. However, we cannot exclude the easier access to analytical instrumentation to determine mycotoxins together with the evolution of the analytical methods *per se* as a plausible contribution to this shift.

The results of the most recent mycotoxin occurrence surveys of aquaculture feeds (Gonçalves *et al.* 2018a; Gonçalves *et al.* 2018; Gonçalves *et al.* 2017; Marijani *et al.* 2017; Nácher-Mestre *et al.* 2015) clearly show an increase in mycotoxin occurrence compared to previous surveys (Alinezhad *et al.* 2011; Almeida *et al.*, 2011; Altuğ and Berklevik, 2001; Bintvihok *et al.* 2003). Unfortunately, it cannot be concluded, from this data, that there is a higher mycotoxin risk now compared to the past. This is because the target mycotoxins analysed in older studies were not the same and sensitivity detection levels and methodologies have since improved significantly. Nonetheless, it was theoretically expected that an increasing level of plant meals in aquafeeds would lead to increased occurrence of mycotoxins in these feeds, which is observable by the most recent occurrence surveys (Gonçalves *et al.* 2018; Gonçalves *et al.* 2018; Gonçalves *et al.* 2017; Marijani *et al.* 2017; Nácher-Mestre *et al.* 2015).

Besides the increasing mycotoxin occurrence and the focus on a broad range of mycotoxins, several other important conclusions can be taken from the studies summarized in Table 1. A key aspect is the regional differences in mycotoxin occurrence reported and the correlation between fungi contamination and the presence of mycotoxins. The presence of molds in a

fish feed is the first indication that something is wrong with its hygiene. There are several reasons why feeds get moldy, from improper storage conditions (high humidity, high variations in temperatures leading to condensation, etc) to poor manufacturing process (e.g., insufficient drying time, lack of perservatives/anti-molds, etc). Fungi contamination can also originate from inappropriate selection of ingredients, which can carry fungi spores that are resistant to extrusion/pelleting, having the capacity to germinate afterwards (due to improper storage or poor manufacturing processes).

While the presence of fungi might be a direct risk for the host, e.g., *Fusarium oxysporum* and *Fusarium solani*, known as opportunistic pathogens for fish and shrimp (Hatai *et al.* 1986; Lightner, 1996; Ostland *et al.* 1987; Souheil *et al.* 1999), and an indirect risk which reduces the palatability and therefore intake of the feed, its presence does not necessarily correlate with the presence of the toxin producer mold and *vice-versa* (Alinezhad *et al.* 2011; Greco *et al.* 2015). On the other hand, mycotoxins produced on crops in the field will remain in raw materials, even after processing, due to their heat stability (Pitt, 2014), while fungi will be destroyed due to high temperatures. For example, *Fusarium spp*. are field fungi usually lacking the ability to grow on dry feed. However, the toxins produced by these fungi species (e.g., DON, FUM) will remain stable on the plant raw materials used to manufacture aquafeeds, and in some cases, even be redistributed and concentrated in certain milling fractions (Cheli *et al.* 2013) e.g, corn *vs* corn gluten meal (Gonçalves *et al.* 2018a). Mycotoxin redistribution and transfer from crops to aquafeeds has been observed and reported by Gonçalves *et al*. (2018a). While it is not the core of the present review, we need to highlight that, with the exception of AF and OTA, most of the other mycotoxins found in the occurrence surveys and shown in Table 1 are probably due to the use of plant meals rather than mycotoxins being produced during storage. So, the selection and analysis of the plant raw materials selected to manufacture aquafeeds is the first step to minimise mycotoxin accumulation risks in aquafeeds.

The regional differences in mycotoxin occurrence is also an important factor which cannot be overlooked. Fungal growth, and consequently mycotoxin production in crops, is influenced by several factors, with weather conditions being the most important (Miraglia *et al.* 2009; Paterson and Lima, 2010; Paterson and Lima, 2011). Consequently, it could be expected that different regions present differences in mycotoxin contamination patterns, and even within a region, mycotoxin occurrence may vary depending on seasonal conditions.

This is shown by the data reported by Bintvihok *et al*. (2003) in samples from Thailand, which suggests that rainy seasons might be more problematic and therefore should be closely monitored. However, factors such as climate change and the world trade of commodities makes it challenging to estimate the risk of mycotoxins in aquaculture finished feeds. For example, as reported by Gonçalves *et al*. (2018), higher levels of FUM in European finished feeds compared to SE Asia samples cannot be easily explained and therefore a better understanding on the origin of sourced ingredients is necessary. The increasing globalisation of trade commodities and incorporation of imported raw materials into aquafeeds exposes the industry to the potential risk of mycotoxins, which are sometimes not even common for the region (not the case in that particular study). Therefore, mycotoxin contamination needs to take into account the globalisation of raw materials, which could already have significant levels of mycotoxins together with the monitoring of finished feeds.

EMERGING MYCOTOXINS

Emerging mycotoxins are a class of mycotoxins which its occurrence in feed and food commodities has been increasing only recently (Kovalsky *et al.* 2016) and which may represent a potential toxicity towards animals and humans. The presence of these mycotoxins also produced by *Fusarium* spp. (as are DON, FUM and ZEN described previously) is expected to increase due to climate change (Miraglia et al. 2009; Paterson and Lima, 2010; Paterson and Lima, 2011). However, quantitative estimates of their occurrence are scarce, especially in aquaculture feeds. While for trichothecenes, data on its toxicity, occurrence, and contamination levels are available, reported in previous section, for other metabolites also produced by *Fusarium* spp., such as moniliformin (MON), fusaproliferin (FUS), beauvericin (BEA) or enniatins (ENNs), limited information is available. Moreover, the typical *Fusarium* mycotoxins (DON, FUM and ZEN) are legislated for certain levels in feed commodities, however, for this new diverse group of “emerging toxins” e.g., MON, FUS, BEA and ENNs, legislation is scarce (Kovalsky et al., 2016). Besides that, the effects of these mycotoxins on aquaculture species is still relatively unknown (Gonçalves et al. 2018; Jestoi, 2008; Nguyen et al. 2003; Tuan et al. 2003; Yildirim et al. 2000). Generally, is observed that, regulated mycotoxins, i.e., FUM, DON and ZEN occurrence levels in feeds are still higher than these emerging mycotoxins (Kovalsky et al. 2016). However, Tolosa et al. (2013) identified several enniatins (ENNs; ENA1, ENB and ENB1) in seabream, seabass, tilapia and panga

tissues from commercialized aquaculture fishes. To our knowledge, Tolosa et al. (2013) study is the first of its kind and highlights for the need to better understand mycotoxin carry-over beyond the typical *Fusarium* spp. mycotoxins. This topic will be further discussed in section "Data obtained from commercially sourced aquaculture products".

CARRY-OVER OF MYCOTOXINS

Bioaccumulation of mycotoxins from feed to animal food products might represent a direct risk to human health (CAST 2003). Mycotoxin bioaccumulation in livestock is well investigated (I. Völkel *et al.* 2011; Leeman *et al.* 2007) and the risk to humans is currently being evaluated by the European Food Safety Authority (EFSA) for several mycotoxins (AF, OTA, ZEN, DON, FUM, T-2 and HT-2). Bioaccumulation of mycotoxins in poultry, swine and cows is managed by direct regulation of mycotoxins in animal feed (EC, 2006; EFSA, 2004a; EFSA, 2004d; EFSA, 2004c; EFSA, 2005; EFSA, 2011; EFSA, 2013). While regulatory limits have been put in place for AFs (), only guidance values are available for DON, OTA, FUM and zearalenone (ZEN; EC, 2006). This is because feed does not represent a direct risk for human health and because carry-over of these mycotoxins in terrestrial animals is expected to be low (EC. 2006).

Currently, no regulations or guidelines exist in order to avoid deposition of mycotoxins in farmed fish or shrimp, with the exception of fumonisins (FB1 + FB2 = 10 mg kg^{-1}; EC. 2006). Moreover, it is not taken into consideration that carry-over mechanisms in aquaculture farmed species might be different from terrestrial livestock species. Generally, the possibility of mycotoxin bioaccumulation/biomagnification through the food chain due to the use of mycotoxin contaminated non-plant origin ingredients such as animal by-products (e.g., shrimp head meal or chicken droppings (further discussed in section "Carry-over data obtained from feeding trials"; "Aflatoxins")) or non-typical mycotoxin contaminated ingredients (e.g., fishmeal), is not taken into consideration and will be addressed during this review.

Bioaccumulation of mycotoxins in aquaculture seafood products is not widely reported and consequently not regulated. This section will focus on documented peer-reviewed mycotoxin carry-over studies focussed in aquaculture species. Existing literature is reviewed, calculating transfer factors when the available data allows it, in order to compare

bioaccumulation risks (Leeman *et al.* 2007). The transfer factor is expressed as the concentration of mycotoxin in animal tissues (μg kg^{-1}) divided by the concentration of the same mycotoxin in animal feed (μg kg^{-1}).

Carry-over data obtained from feeding trials

The present section intends to give an overview of studies reporting the carry-over of mycotoxins from feed to animal tissues, assessed in feeding trials with supplemented mycotoxins in feed. We calculated transfer factors for carry-over of mycotoxins from feed to eggs, whole milk, meat and edible offal as calculated by Leeman *et al*. (2007) (Table S1). The data presented by Leeman *et al*. (2007) covered 250 references resulting in a comparison of 3624 transfer factors from livestock species (cattle, poultry, pig, sheep, goat, rabbit, pheasant, turkey, duck and quail). These authors took into account the carry-over of AFs (AFB_1, AFB_2, AFG_1 and AFG_2), DON, OTA, T-2 and ZEN. Leeman *et al*. (2007) reported average transfer factors, ignoring the differences in different mycotoxin kinetics as well as the different metabolism capacity of animals. Nonetheless, the information gathered has a high relevance and allows a first comparison between transfer factors in aquaculture-farmed species *versus* livestock.

Aflatoxins (AFs)

Aflatoxin bioaccumulation from feed to animal tissues is well documented for aquaculture species. A total of 19 studies have evaluated the presence of AFs in fish and crustacean tissues after being fed a certain amount of this same mycotoxin (Table 2).

The first study (Suzy *et al.* 2017) reported in Table 2 raises an interesting and not yet discussed point about the occurrence of mycotoxins in feed conveyed from animal by-products and not necessarily from plant meals. Suzy *et al.* (2017) reported that with increasing aquaculture production in Africa, in this case the West Cameroon region, feed ingredients are a serious limitation to the sustainable growth of the aquaculture sector. The author reported that due to the good protein content, chicken droppings were being used as an ingredient in the local fish food or as direct feed, despite its contamination with AF's. Suzy *et al.* (2017) reported that after feeding African sharptooth catfish (*Clarias gariepinus*) with 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 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

found that both AF levels led to accumulation in the liver and muscle, however, in the liver, AFs were found earlier (six weeks post-intake) than in the muscle (only after 12 weeks). The intake period is an important factor to take into consideration as shown by Mahfouz and Sherif (2015), and equally important would be to establish suitable depuration periods for the different mycotoxins. If feasible, adequate fasting periods before harvesting which currently vary from species to species could be set according to mycotoxin tissue levels. Despite using a considerably high range of AFB_1 levels in his study, Deng *et al*. (2010) observed during a 20 week trial, that even relatively low AFB_1 levels (85 μg kg^{-1}) could lead to a significantly high accumulation of AFB_1 in the liver after 20 weeks of ingestion (AFB_1 in the liver after 20 weeks = 30 μg kg^{-1}; Table 2). In short exposure periods to AF (30 days), Abdel Rahman *et al*. (2017) observed that the intake of 200 μg kg^{-1} of AF accumulated in the liver and muscle at 5 ± 0.5 and 3.7 ± 0.1 μg kg^{-1}, respectively. This might suggest a certain incapability to metabolize AF.

Other studies also performed in tilapia (*Oreochromis niloticus*) (Ayyat *et al.* 2013; Salem *et al.* 2009; Selim *et al.*, 2014), support the previously reported studies, but show a tendency for a higher accumulation of AFs in muscle (Table 2), which could be related to the smaller size of the tilapias used (7 to 15 grams). For example, Selim *et al*. (2014) reported the deposition of 90 μg kg^{-1} of AFs in the muscle after feeding tilapia (15 ± 2 g) with 200 μg kg^{-1} of AF for ten weeks. Likewise, the Ayyat *et al*. (2013) and Salem *et al*. (2009) studies that used fish with an initial weight of 7.3 g and 10 g, respectively, also showed high values of AFs in the muscle (78.33 μg kg^{-1} and 99.48 μg kg^{-1}, respectively). In comparison, in the study by Mahfouz and Sherif (2015) that used fish with an initial weight of 35 g, intake of 100 μg kg^{-1} AF over 12 weeks led to a lower accumulation of AF in the muscle (0.05 μg kg^{-1}). This tendency for higher AF deposition in younger animals seems to be further confirmed by Hessein *et al*. (2014), where after feeding tilapias of 7.3 grams for 98 days with 250 μg kg^{-1} AF, an AF deposition of 101.7 μg kg^{-1} was found. This means a TF of 0.407 that, together with data reported by previous authors (Salem *et al*. 2009, Selim *et al*. 2014), have relatively high TFs for muscle and are only comparable to livestock edible offal (Table S1).

Finally, Hussain *et al.* (2017) showed a high deposition of AF in tilapia muscle, however, the levels of mycotoxins used in this trial (2000 to 4000 μg kg^{-1}) are unlikely to be found in aquafeeds although TFs calculated for AF deposition in the liver are in line with the other studies. The only trial with red tilapia (*Oreochromis niloticus x O. mossambicus*), (Usanno *et*

al. 2005) reported no detectable levels of AF in tilapia tissues, after being fed AF levels ranging from 50 to 2500 μg kg^{-1}.

The deposition of AFs in the liver and muscle of Gibel carp (*Carassius gibelio*) are similar to the levels reported for Nile tilapia (Huang *et al.* 2011).

Lopes *et al*. (2009) reported the deposition of AFs in the liver and muscle in Jundiá (*Rhamdia quelen*) fed low (41.90 and 204 μg kg^{-1}) and high (350, 757 and 1177 μg kg^{-1}) AF levels for 45 and 35 days, respectively. Focusing on lower AF levels, as they are whithin the observed AF's occurrence levels in aquafeeds, 41.90 μg AF kg^{-1} feed led to the deposition of 1 μg kg^{-1} in the muscle and 204 μg kg^{-1} of AFs led to the deposition of 6.1 μg kg^{-1} AFs. These bio-accumulation level of AFs leads to TFs of 0.02, which is comparable to the level of accumulation on livestock edible offal's ((Leeman et al. 2007); Table S1)

Lambari fish (*Astyanax altiparanae*), a native central/south American small fish (10-15 cm length and 60 g), has been seen as a potential aquaculture species for rural population in Brasil. Michelin *et al*. (2017) reported lambari fish as highly prone to AF deposition in the liver and muscle. After lambari fish were fed 20 kg^{-1} of AFs for 120 days, deposition of AFs in 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 μg kg^{-1} (TF 4.86). This level of bio-accumulation in the liver is higher than the bioaccumulation of highly liposoluble mycotoxins in terrestrial animal fat ((Leeman *et al.* 2007); Table S1). Such AFs levels in this species could be particularly challenging as these fish are normally eaten as snacks, i.e., the entire fish is deep-fried, dried and/ or salted.

Reports of AF carry-over in shrimp are limited to three studies performed in black tiger shrimp (*Penaeus monodon*). Two of these studies (Bintvihok *et al.* 2003; Bautista *et al.* 1994) did not find any AF residues after feeding shrimps with different AF concentrations (5 to 200 μg kg^{-1}) for 10 and 62 days, respectively. In contrast, Boonyaratpalin *et al*. (2001) found AF residues in cephalothorax and in muscle, after feeding the shrimps AFB_1 levels ranging from 50 to 2500 μg kg^{-1} with TF values ranging from 0.006 to 0.052. Contextualizing the AF contamination levels found in feed around SE Asia (< 500 μg kg^{-1}; (Fegan and Spring, 2007; Gonçalves *et al.* 2018a; Gonçalves *et al.* 2018; Gonçalves *et al.* 2017)) with the Boonyaratpalin *et al*. (2001) study, shrimps fed AFB_1 levels of 50 and 100 μg kg^{-1} led to considerably high AF deposition in head and shell (2.6 and 3.5 μg kg^{-1} AFB_1, respectively) and in muscle (13 and 14.2 μg kg^{-1} AFB_1, respectively), after four weeks of AFB_1 intake. For the same intake amounts (50 and 100 μg kg^{-1} AFB_1), AFB_1 deposition levels in head/shell and

muscle samples decreased over time (after six weeks; Table 2). This might suggest a certain capacity to eliminate or metabolize AFB_1.

Ochratoxins (OTA)

Ochratoxin bioaccumulation studies in aquaculture-farmed species are very scarce. The most comprehensive study was carried out by Bernhoft *et al*. (2017) in Atlantic salmon (*Salmo salar*). Bernhoft *et al*. (2017) studied the deposition of OTA in liver, muscle, kidney and skin samples after feeding salmon with 800 or 2400 μg kg^{-1} of OTA for eight weeks. Deposition of OTA in kidney and skin samples was not detected (except in kidney for high intake dosage after eight weeks, Table 3). In muscle samples, OTA levels were under the limit of quantification. Major deposition was observed in the liver, however, a bioaccumulation over the exposure period was not found, with the highest OTA deposition peaking after three weeks (both for ingestion of 800 and 2400 μg kg^{-1} OTA). This suggests that Atlantic salmon might have the ability to eliminate OTA. Previously, OTA deposition in salmonids (rainbow trout (*Oncorhynchus mykiss*)) was investigated by Fuchs *et al*. (1986) where the deposition of OTA in several organs (Table 3) was analysed up to eight weeks after an intravenous injection of OTA (0.160 μg kg^{-1}). Authors observed that OTA deposition in the kidney and bile was persistent during the whole trial, also suggesting the action of the kidney in detoxification mechanism of OTA. The only study reporting carry-over of OTA in shrimp (*Penaeus monodon*) was by Supamattaya *et al*. (2005a), which did not detect OTA deposition in tissues after feeding shrimps with OTA levels ranging from 100 to 1000 μg kg^{-1}. However, the limit of detection given in the manuscript (44,000 μg kg^{-1}) seems to be particularly high for HPLC, suggesting a possible error in the units reported.

Deoxynivalenol (DON) and fumonisins (FUM)

Deoxynivalenol and/or FUM bioaccumulation data in aquaculture species is summarized in Table 4. Similar to OTA, DON and FUM carry-over effects in aquaculture-farmed are scarce. In Atlantic salmon (*Salmo salar*), two studies are available (Bernhoft *et al.* 2017 and Nácher-Mestre *et al.* 2015). Bernhoft *et al*. (2017) fed salmon with 2000 and 6000 μg kg^{-1} DON over the course of eight weeks and sampling liver, muscle, kidney and skin at three, six and eight weeks. The authors observed that both exposure dosages (2000 and 6000 μg kg^{-1} DON) led

to DON deposition in the liver and muscle at all sampling points, except for the higher dosage at the last sampling point (eight weeks), at which DON was found in all sampled tissues (Table 4). In the case of the study performed by Nácher-Mestre *et al*. (2015), Atlantic salmon were fed lower levels of mycotoxins, however, with multi-occurrence. The three diets were mainly formulated with DON and FUM, but also minor levels of T-2 and 15-AcDON (Table 4). Salmon fed for six months with testing diets did not show detectable levels of DON and FUM in the tissues studied. The same authors (Nácher-Mestre *et al.* 2015) also studied bioaccumulation of mycotoxin co-occurrence (DON, 15-AcDON and FUM) in Gilthead sea bream (*Sparus aurata*) at two levels for 8 months. The authors did not observe mycotoxin deposition in muscle samples.

In common carp (*Cyprinus carpio*), Pietsch *et al.* (2014) observed that after feeding fish with 352, 619 and 953 µg kg^{-1} DON for four weeks, minor deposition of DON was observed in the muscle (Table 4). Interestingly, after the four weeks of DON exposure, fish were fed a non-contaminated diet for a period of two weeks and DON levels in the muscle were re-analysed. At the lower DON intake level (352 µg kg^{-1}), DON level in the muscle was higher after the depuration period (1.4 µg kg^{-1}) when compared to the level found at the end of feeding trial (eight weeks; 0.6 µg kg^{-1} DON). At the medium DON intake level (619 µg kg^{-1}), after the recovery period, a level of 0.7 µg kg^{-1} DON was still found in the muscle, and at the higher level, however, no DON was detected after the recovery period.

In shrimps, two studies are available (Supamattaya *et al.* 2005b and Trigo-Stockli *et al.* 2000) Table 4), in which both reported that DON was not detected in the muscle. Supamattaya *et al.* (2005b) drew its conclusion after feeding black tiger shrimp black (*Penaeus monodon*) with 500, 1000 and 2000 µg kg^{-1} DON for eight weeks. Trigo-Stockli *et al.* (2000) conducted its study using Pacific white shrimp (*Litopenaeus vannamei*), fed with 200, 500 and 1000 µg kg^{-1} DON for 16 weeks.

Zeralenone (ZEN)

Zearalenone (ZEN) is a regular contaminant of cereal crops worldwide, and being a phytoestrogenic compound (Diekman and Green, 1992), is mainly responsible for estrogenic agonist related effects (Marasas, 1991). As a hormone mimicking substance, ZEN 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.

From these studies, it is possible to conclude that AFs might represent a serious risk for human consumption, especially in cases where fish are eaten as a whole. In general, transfer factors are quite high for these aquaculture species, being comparable with transfer factors for eggs, whole milk and in some cases for edible offal's or fat of livestock provenience.

In the case of European seabass, mycotoxin levels tested by El-Sayed and Khalil (2009) (18 µg kg^{-1}), which is a mycotoxin level very plausible to be obtained in commercial diets led to 4.25 ± 0.85 µg AFB_1 kg^{-1} in the muscle,. As shown by Altuğ and Berklevik (2001) (Table 1), of the 170 samples collected in Turkey, which is the main EU seabass producer, 105 samples were contaminated with AFs at levels higher than 20 µg kg^{-1}. Regarding hybrid sturgeon (*Acipenser ruthenus*), there is no available mycotoxin occurrence data for this species, even in regions where it is predominantly produced. However, in-feed concentrations tested by Rajeev Raghavan *et al*. (2011), which led to the accumulation of AF in the muscle and liver, seem realistic (40 to 80 µg AFB1 kg^{-1}) and therefore further research should be carried out to determine mycotoxin levels in feed for this species and AF accumulation in eggs (caviar).

Carry-over effects on Nile tilapia are well described. Taking into account the available occurrence of AF in tilapia producing countries, i.e., Brasil (Barbosa *et al.* 2013), S/ SE Asian countries (Fegan and Spring, 2007; Gonçalves *et al.* 2018a; Gonçalves *et al.* 2018; Gonçalves *et al.* 2017) and Africa (Marijani *et al.* 2017) together with bioaccumulation studies, carry-over of AF in Nile tilapia might represent a challenge worth of further investigation. From the previously cited studies, it is also important to highlight that exposure period is an important factor to take into consideration. Chronic exposure to low AF levels (AF = 85 µg kg^{-1} for 20 weeks) could lead to a significantly high accumulation in the liver (AF in the liver after 20 weeks = 30 µg kg^{-1} (Deng *et al.* 2010)). However, short exposure periods should not be undervalued, as periods as short as 30 days can lead to considerable AF deposition in the liver and muscle (Abdel Rahman *et al*. 2017).

Aflatoxin carry-over studies in shrimp are more limited than in fish species. Furthermore, the information available is contradictory, as two studies (Bintvihok *et al.* 2003 and Bautista *et al.* 1994) did not find any AF residues in tiger shrimp muscle while Boonyaratpalin *et al.* (2001) found AF bioaccumulation in head/shell and in the muscle. Results suggested a minor bioaccumulation over time (TFs; Table 2), highlighting a certain capacity to eliminate or metabolize AFB_1. However, levels of AF found in the muscle (13 µg kg^{-1} AFB_1) after feeding 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

tested were within the occurrence values reported in European aquafeeds (Gonçalves *et al.* 2017; Gonçalves *et al.* 2018), however, occasional high occurrences of DON and/or FUM should not be ignored (e.g., FUM occurrence reported by Gonçalves *et al.* (2018)), as shown previously, levels up to 2000 µg kg^{-1} can lead to DON deposition in the muscle.

Contrary to Atlantic salmon, in common carp (*Cyprinus carpio*), Pietsch *et al.* (2014) showed that levels as low as 352 µg kg^{-1} DON can lead to a minor deposition of DON in the muscle (Table 4). The author described that total DON elimination from the muscle is a relatively long process, taking more than two weeks after stopping DON intake. Information about the complete elimination of DON is very important, as a fasting period before harvesting may be used to guarantee that DON or any other mycotoxin is eliminated during this period. However, in the study reported by Pietsch *et al*. (2014), the elimination period of DON in carp may be longer than the fasting period, which is normally 24 to 48 hours before harvesting. The study by Pietsch *et al*. (2014) highlighted that mycotoxin absorption, distribution, metabolism, and excretion (ADME) is entirely dependent on species, and data or conclusion extrapolations between species should be avoided. *Fusarium* mycotoxins (e.g., DON and FUM) are frequently present in plant commodities used for general aquaculture species, and taking into account the possible ADME differences depending on species and even on development stages, it would be very important to better understand the potential carry-over in the most important aquaculture species, giving a special emphasis to mycotoxin co-occurrence.

Despite the low number of studies on DON and FUM carry-over, apparently, its deposition in tissues seems to be very limited. However, its occurrence is frequent and due to its apparently long elimination period (generally higher than fasting period before slaughter, for the study species), its carry-over risk in aquaculture-farmed species should be better evaluated. Comparing TFs obtained from Atlantic salmon and common carp, it seems that they are in line with the TFs of eggs, whole milk or meat (Table S1, (Leeman *et al.* 2007)).

Is also important to highlight that the species investigated so far are cold/temperate water species. It is essential to increase the knowledge on the possible carry-over of *Fusarium spp.* mycotoxins in tropical species. Especially high value species, normally exported, such as Pacific white leg shrimp, whose feeds have been identified recently as being contaminated 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

Table 6 documents mycotoxin occurrence in commercially sourced aquaculture products. Evaluating the occurrence of mycotoxins directly in fish/shrimp products from aquaculture provenience obtained from commercial farms or local supermarkets is a good strategy to evaluate the potential risk of mycotoxin carry-over from feeds to fish/shrimp edible products. Tolosa *et al.* (2013) analysed several samples (n = 19) of fish from aquaculture and wild fishery provenience bought locally in Spain. The author analysed samples for the presence of beauvericin (BEA) and enniatins (enniatin A (ENA), enniatin A1 (ENA1), enniatin B (ENB) and enniatin B1 (ENB1)). As expected, no mycotoxins were detected in the wild fishery samples. ENA and BEA were also not detected in the aquaculture samples. However, ENA1, ENB and ENB1 were detected in most of aquaculture samples (Table 6). Detecting enniatins in aquaculture foods might lead us to two hypothesis. First, that other *Fusarium* mycotoxins (FUM, DON and ZEN mainly) were probably at even higher concentration levels and are not reported as they were not analysed. The second hypothesis is the fact that ENNs might be more easily deposited in the muscle compared to DON/FUM, even if present at lower levels in aquafeeds. As it is known that ENNs normally occur together with the main *Fusarium* mycotoxins (FUM, DON), it would also be important to study if this synergistic presence in the tissues might lead to increased deposition of certain mycotoxins or metabolites. While it is difficult to evaluate the importance of detecting ENNS in aquaculture foods, these results highlight the need to better study the adverse effects of dietary mycotoxins on fish health and welfare, and consequently carry-over risks. There is the need to perform studies for the main EU farmed fish species in order to establish acceptable feed mycotoxin levels for farmed fish (for both fish and consumer safety), but also to actively survey possible mycotoxin deposition in imported aquaculture foods.

Woźny *et al.* (2013) analysed ZEN in rainbow trout from farms based in the north-eastern region of Poland. ZEN was present at non-quantifiable levels (<2.0 μg kg^{-1}) in most of the tissues analysed (intestine, liver and ovary) and detectable at quantifiable levels in the muscle and surrounding water. From 2013 to 2015, Woźny *et al.* (2017) surveyed ovary, oocytes and salted roe samples from different fish species collected directly at hatcheries or bought in supermarkets. The authors analysed the samples for the presence of ZEN, α-ZEL and β-ZEL. Generally, in most of the samples analysed mycotoxins were below the detection limits (LOD for ZEN, α-ZEL, and β-ZEL were 5.0, 3.0, and 12.0 μg kg^{-1}, respectively). The exceptions were α-ZEL in ovary samples (14.5 μg kg^{-1}) of *Oncorhynchus mykiss* and α-ZENL

also in ovary samples (12.6 µg kg^{-1}) of *Salvelinus fontinalis* both sampled in 2014. The studies reported by Woźny *et al*. (2013; 2017) are also extremely important and highlight the need for guidance values for the amount of ZEN in aquafeeds for fish health and reproductive performance, but also to avoid carry-over risk to human consumers.

Although it did not investigate fish originating from aquaculture, it is important to highlight the recent study published by Slawomir Gonkowski et al., (In Press). Slawomir Gonkowski *et al*., (In Press) evaluated the deposition of ZEN in sun-dried kapenta fish, which is one of Zambia's major staple foods. This small planktivorous fish is caught in Lake Kariba, sun-dried and sold in local markets. Although the source of the ZEN deposition is not known, the study revealed that levels of ZEN in sun-dried kapenta fish fluctuated from about 27 µg kg^{-1} to above 53 µg kg^{-1}. Occurrence of ZEN in sun dried kapenta fish, highlights that carry-over guidelines cannot be assumed only for farmed animals as species and local consumption habits pose mycotoxin-related risks to wider seafood products.

Further considerations

Despite the effort to document mycotoxin occurrence in aquaculture feeds, we are still far from having a good overview on this topic. One of the big challenges is the large number of aquaculture-farmed species, and the impossibility to extrapolate occurrence results from one species to another. Moreover, different species, even in same trophic level, tend to be fed with different raw materials based on local availability and price. This leads to a huge difficultly in having a good overview of mycotoxin occurrence for all aquaculture species or even for a certain region. Nevertheless, knowledge about mycotoxin occurrence in aquaculture commodities could increase significantly if we could better use the available occurrence data from livestock. Surveys on mycotoxin occurrence in plant meals worldwide are frequently available, and this information can be used, at least, to theoretically model the risk of plant feedstuffs included in aquafeeds. However, a fundamental problem is the lack of detailed labelling information regarding ingredient inclusion by (percentage) weight. Therefore, an improvement in labelling policy would help to identify and map sources of mycotoxin inclusion in animal feed, avoiding extra costs for testing mycotoxin levels in finished feeds. Therefore, a close collaboration with the agricultural and livestock sectors to understand the occurrence of mycotoxins in plant meals, might also help to improve our knowledge on mycotoxin conveyance to aquafeeds.

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

To our knowledge not yet addressed in an aquaculture context, is the potential for mycotoxins to contaminate water, especially taking into account water stable mycotoxins and closed or semi-closed aquaculture systems. Bucheli *et al.* (2008) evaluated the presence of ZEN and DON in Swiss rivers, confirming the presence of both mycotoxins at levels ranging 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 possibility of mycotoxins as water contaminates, which in the aquaculture context might be extremely relevant. The mycotoxin leach from aquafeed to system water, especially of highly water-soluble mycotoxins in slow feeding species, e.g., DON and FUM in shrimp feed, and the water stability of excreted mycotoxins and metabolites, which might have potential to accumulate, especially in low water hydrodynamics and low renovation rate aquaculture systems, should be urgently addressed.

CONCLUSION

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

Fusarium metabolites might be more prone to bioaccumulation than the most common frequently analysed *Fusarium* mycotoxins. The presence of enniatins in aquaculture foods highlights the need to understand its potential impact to human food safety.

Regarding ZEN, the potential for deposition in the ovaries and to a lesser extent in oocytes was shown. For the studied species, ZEN can reach considerable levels in the ovaries. No studies are available yet for tropical species. It would be important to investigate whether carry-over of ZEN to ovaries occurs in tropical species as well, as for many of these species, gonads are considered gourmet snacks, representing a direct risk to human health.

While there are many important aquaculture species not investigated yet, it is clear that some mycotoxins are prone to deposition in the tissues of certain aquaculture species. It needs to be considered that in aquaculture species, mycotoxin biotransformation and tendency for deposition in tissues varies greatly depending on factors such as development stage, sex, exposure period and rearing environment.

Due to the use of increasing levels of plant meals in aquafeeds and together the possible mycotoxin increase due to climate change, it is essential to develop more studies on the impact of mycotoxins and metabolites on farmed species with consequent risk assessment of food safety from mycotoxin-contaminated aquafeeds.

Regulation limits for mycotoxins in feeds might need to take into account particular aquaculture species or the sector as a whole. Mycotoxin limits need to take into consideration animal health and welfare but also human health. Particular attention needs to be focused on aquaculture edible tissues and regional guidance limits should be advised depending on local mycotoxin occurrence and the edible tissues consumed. Risk assessment of imported aquaculture foods needs to take into account the mycotoxin occurrence, especially in those products imported from highly mycotoxin contaminated regions, or regions known to use potentially contaminated animal by-products.

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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 (µg 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 µg kg^{-1} AFB_1 n = 21 -> 30 to 40 µg kg^{-1} AFB_1 n = 2 -> 60 to 120 µg 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 µg kg^{-1} n = 85 = 21.2 to 42.4 µg kg^{-1} n = 22 = 5.0 to 20.0 µg 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 µg kg^{-1} AFB_1	HPLC	▪ High concentrations of AFB_1 in fishmeal ($\bar{x}$= 67.35 µg kg^{-1}) and soybean meal ($\bar{x}$= 30.88 µg 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 µg g^{-1} for ELISA (FB_1) ▪ 0.003 and 0.005 µg 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; $\bar{x}$ = 236.18 ZEN = 3-511; $\bar{x}$ = 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; $\tilde{x}$ = 2.82 OTA = 3.5 – 5.0 $\tilde{x}$ = 5.26 T-2 = 50 – 105.99; $\tilde{x}$ =70.08 FUM = 190 -222; $\tilde{x}$ = -- DON = 150 – 210; $\tilde{x}$ =230 ZEN = 20.04 – 159.76; $\tilde{x}$ = 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: $\bar{x}$ = 51.83; Max = 220.61; (21/31) [A]ZEN: $\bar{x}$ = 60.41; Max = 232.88; (18/31) [A]DON: $\bar{x}$ = 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

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

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.
General abbreviations: $\bar{x}$ = 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
Mycotoxins: 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.
Regions: 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 (µg 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] µg AFB_1 kg^{-1}	M[1] = 0.05±0.12 µg AFB_1 kg^{-1} M[2] = 0.08±0.10 µg AFB_1 kg^{-1} M[3] = 0.08±0.12 µg 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 µg AFB_1 kg^{-1} ▪ Catfish fed 10 µg 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 µg kg^{-1}	[#2]M = 4.25 ± 0.85 µg 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] µg AFB_1 kg^{-1}	L[1-5] > 5 µg AFB_1 kg^{-1} M[5] = 2.35 µg 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 ruthenus x 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±0.5 µg AFB_1 kg^{-1} M^{1} = 3.7±0.1 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[2]; 793[3] and 1,641[4] µg kg^{-1}	$Y^{t1\text{-}tf;0\text{-}4}$ 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	$Y^{t1\text{-}tf;0\text{-}4}$ 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 kg^{-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±1.32 µg kg^{-1}	M^{2} ~ 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} $L_{1,t2}$ = 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

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

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] ng g^{-1} [HP]m= 48.61±3.13[5] ng 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.

General abbreviations: 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

Tissue abbreviations: 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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