Research Article

Detection of Mycotoxins in Imported Fish Feeds Analyzed by the Quality Control Laboratories, Wasit Province, Iraq

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Abstract | Imported fish feeds entering Iraq through the Al-Shaib border crossing were evaluated for compliance with national mycotoxin limits. Samples were collected under the supervision of the Animal Resources Department and analyzed at the Wasit Quality Control Laboratory from June to September using Veratox® quantitative ELISA kits specific for each mycotoxin. All samples contained detectable but very low levels of the target mycotoxins, with mean concentrations ranging from 0.286 to 0.857 ppb for aflatoxins, 1.378 to 15.39 ppb for T 2/HT 2, and 0.150 to 0.312 ppb for ochratoxin A, which are well below the Iraqi permissible limits of 20, 100, and 5 ppb, respectively. Some seasonal variation was observed, including slightly higher aflatoxin and ochratoxin A concentrations in July and a transient decrease in T 2/HT 2, but none approached regulatory thresholds. Overall, these findings indicate that imported fish feeds examined during the study period complied with national mycotoxin standards, while underscoring the importance of routine border control monitoring to protect fish health, aquaculture productivity, and public health.

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Introduction

Fish production in aquaculture has increased rapidly over recent decades, leading to a parallel rise in the number of farmed fish and, consequently, a growing demand for formulated feeds (Munguti *et al*., 2024). To meet this demand, aquaculture increasingly relies on compound feeds rich in plant-derived ingredients, which are particularly prone to fungal contamination and, therefore, to mycotoxin occurrence, as a result, mycotoxins are now commonly detected in such feeds, especially those based on cereals and oilseed by-products (Pietsch, 2020).

The problem of mycotoxin contamination in feed ingredients is believed to have worsened due to climate change and the globalization of trade, which facilitates the spread of toxigenic fungal species and often leads to higher contamination levels in grains (Dobolyi *et al*., 2013). Several mycotoxins also show considerable stability during heat treatment, and improper storage of both raw materials and finished feeds further contributes to increased levels of these toxins in fish feeding systems (Pietsch, 2020). Mycotoxin production depends on the toxigenic capacity of specific fungi and on environmental conditions, including physical, chemical, and biological factors (Piotrowska *et al*., 2013; Pietsch, 2020). Moreover, processing steps such as cleaning, sorting, grinding, and thermal treatment can influence fungal growth and mycotoxin levels in final products. At the same time, while contamination often starts in the field and may be aggravated by inadequate storage (Piotrowska *et al*., 2013).

The most important mycotoxins found in feed ingredients due to their known toxicity and frequent occurrence at high concentrations include aflatoxin B1 (AFB1), deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEN), ochratoxin A (OTA), T-2 toxin (T-2), fumonisin B1 (FB1), moniliformin (MON), enniatins (ENNs), and beauvericin (BEA) (Czerwiecki *et al*., 2002; Pietsch, 2020). Mycotoxins in contaminated feeds can severely impair fish health and performance, causing reduced growth, increased mortality, immunosuppression, and damage to the liver, spleen, and kidneys, as well as DNA damage and carcinogenic effects; Mycotoxins present in contaminated feeds can severely impair fish health and performance and, in some cases, may also be transferred from feed to edible tissues, making fish consumption a potential route of mycotoxin entry into the human food chain (Pietsch, 2020; Oliveira and Vasconcelos, 2020). In addition, mycotoxin contamination can markedly reduce animal productivity (Abdulhameed *et al*., 2023), underlining the need to control aflatoxin levels in feeds, apply good agricultural practices, and implement effective post-harvest and storage strategies.

Accordingly, there is a need to monitor mycotoxin contamination in imported aquafeeds under local conditions. In this context, monitoring mycotoxin contamination in imported aquafeeds under local climatic and storage conditions is crucial, the present study aimed to qualitatively and quantitatively detect the major mycotoxins aflatoxin, ochratoxin A, and T-2/HT-2 toxins in imported fish feeds entering Iraq through the Al-Shaib border crossing, to compare the detected levels with international maximum permissible limits, and to provide reliable laboratory data to support regulatory decisions by the Quality Control Laboratories, Wasit Branch.

Materials and Methods

The study was conducted at the Quality Control Laboratory, Wasit Branch, under the supervision of the Director of the Animal Resources Department. Fish feed samples were collected at the Al-Shaib border crossing upon arrival of imported feed trucks, after recording all relevant shipment information, including producing company, product details, and truck identification numbers (Figure 1).

Figure 1: *Collection of representative fish feed samples from imported feed trucks at the Al-Shaib border crossing for subsequent mycotoxin analysis.*

The collected samples were then transported to the Quality Control Laboratory in Wasit to evaluate their suitability and freedom from mycotoxins. At the laboratory, samples were analyzed for aflatoxins, ochratoxin A, and T-2/HT-2 toxins using the specific inspection methods assigned to each test in order to decide whether shipments should be accepted or rejected (Table 1).

All analyses were performed according to the manufacturer's instructions provided in the kit manuals for each specific mycotoxin.

Statistical analysis

The Statistical Analysis System (SAS, 2018) program was used to detect the effect of different months in parameters study. Duncan's multiple range (ANOVA: Completely randomized design-CRD) was used to significantly compare between means at probability on 0.05 and 0.01. The following statistical model:

$$Y_{ij} = \mu + T_i + e_{ij}.$$

Where; Y_{ii} is the observed value of the j-th experimental unit under the i-th treatment (month), μ is the overall mean, Ti is the fixed effect of treatment (month), and e_{ij} is the random error term, assumed to be normally distributed with mean zero and variance σe2.

Results and Discussion

In this study, Table 2 presents the levels of mycotoxins in imported fish feed samples analyzed at the Quality Control Laboratory in Wasit during June, July, August, and September, following activation of the laboratory. All tested samples remained within the permissible limits specified in the Iraqi standard, and the generally low mycotoxin concentrations are likely related to preventive measures applied during feed production, storage, and transport in this period (Abdulhameed *et al.*, 2023). Mean toxin levels were very low, reaching 0.286, 12.84, and 0.195 ppb for aflatoxins, T-2/HT-2 toxin, and ochratoxin A, respectively, which are all below the corresponding maximum limits of <20 ppb, 100 ppb, and 5 ppb set for these toxins.

A slight but statistically significant increase (P≤0.05) in aflatoxin concentration was observed in July and August, with mean values of 0.857 and 0.705 ppb, respectively, compared with June and September. However, these values remained far below the permissible limit of 20 ppb, indicating that aflatoxin contamination in the analyzed samples did not pose an immediate regulatory concern. For T-2/HT-2 toxin, levels decreased significantly (P ≤ 0.01) in July, reaching 1.378 ppb compared with 15.39 ppb in September, which is still below the maximum limit of 100 ppb. However, Ochratoxin A showed a significant increase (P≤0.05) in July (0.312 ppb) compared with September, which recorded the lowest mean level (0.150 ppb); nevertheless, all values remained within the permissible range for ochratoxin A (<5 ppb).

Overall, these findings indicate that, during the study period, imported fish feeds entering through the Al-Shaib border crossing were compliant with current national safety standards for aflatoxins, T-2/HT-2 toxin, and ochratoxin A. Although low-level mycotoxin contamination has been reported to potentially affect fish health under prolonged exposure, the concentrations detected here suggest a low immediate risk from a regulatory perspective, while still underscoring the importance of continuous monitoring and preventive measures along the feed supply chain.

Table 1: *Kit Information for the Detection of Aflatoxin, Ochratoxin A, and T2/HT-2 Toxins in imported fish feeds.*

Test type	Aflatoxins	Ochratoxin A	T-2/HT-2 toxins
Kit name	Veratox® for Aflatoxin	Veratox® for Ochratoxin A	Veratox® for T-2/HT-2 Toxins
Manufacturer	NEOGEN Corporation, Lansing, MI, USA (food safety diagnostic company; https://www.neogen.com)	Same as aflatoxin kit	Same as aflatoxin kit
Principle	Quantitative ELISA (enzyme-linked immunosorbent assay)	Quantitative ELISA	Quantitative ELISA
Storage	2–8 °C (refrigerated)	2–8 °C	2–8 °C
Country of origin	United States of America	United States of America	United States of America

Table 2: *Levels (mean ± SE) of aflatoxins, T-2/HT-2 toxin, and ochratoxin A (ppb) in imported fish feeds from June to September, compared with the Iraqi permissible limits (20, 100, and 5 ppb, respectively).*

Month	Aflatoxins (20 ppb)	T-2/HT-2 (100 ppb)	Ochratoxin A (5 ppb)
June	0.286 ± 0.12 b	12.84 ± 4.35 a	0.195 ± 0.05 ab
July	0.857 ± 0.21 a	1.378 ± 0.48 b	0.312 ± 0.07 a
August	0.705 ± 0.19 a	14.00 ± 3.87 a	0.160 ± 0.06 ab
September	0.629 ± 0.12 ab	15.39 ± 1.62 a	0.150 ± 0.04 b

*Means within the same column bearing different superscript letters differ significantly. * $P \leq 0.05$, ** $P \leq 0.01$ (Duncan's multiple range test).*

In addition to evaluating individual mycotoxin levels, it is important to consider the risks associated with chronic exposure and the co-occurrence of multiple mycotoxins. Although the concentrations detected in the present study were below established permissible limits, prolonged exposure to low levels of mycotoxins may still induce subclinical effects in fish, including reduced growth performance, immunosuppression, and increased susceptibility to diseases (Tacon and Metian, 2008; Pietsch, 2020). These effects are often cumulative and may not be immediately evident, particularly under intensive aquaculture conditions. Furthermore, the co-occurrence of multiple mycotoxins in feed may result in additive or synergistic toxic effects, potentially enhancing their overall impact even when individual toxin levels remain within safe limits (Grenier and Oswald, 2011; Alshannaq and Yu, 2017). Therefore, assessing mycotoxin contamination based solely on individual regulatory thresholds may underestimate the actual risk, highlighting the need for integrated monitoring approaches that consider combined exposure scenarios.

The seasonal pattern of mycotoxin levels is further illustrated in Figure 2. The highest aflatoxin concentrations were recorded in July and August, whereas the lowest mean value occurred in June, with September showing an intermediate level. This pattern may be related to the high temperatures and relatively variable humidity prevailing during midsummer, as such conditions can favor the growth of *Aspergillus flavus* and *Aspergillus parasiticus*, the main aflatoxin-producing fungi (Muga *et al*., 2019). Maintaining storage conditions at a relative humidity below about 60% is generally recommended to minimize aflatoxin contamination and ensure feed safety during storage and transport (Muga *et al*., 2019).

Previous studies have reported that aflatoxin-producing fungi are active over a wide temperature range, roughly between 20–37 °C, with maximum aflatoxin production typically occurring around 25–30 °C when moisture and relative humidity are sufficiently high (Mannaa and Kim, 2017; Mousa *et al*., 2016). These environmental conditions are often encountered in Iraq during the summer months, particularly in inadequately conditioned warehouses or during transport across border crossings, which may help explain the modest increase in aflatoxin levels observed in July and August. In contrast, HT-2/T-2 toxin levels showed a significant decrease in July compared with other months, in agreement with reports suggesting that hot and dry conditions can reduce the prevalence of HT-2/T-2 contamination in stored commodities (Hjelkrem *et al*., 2018).

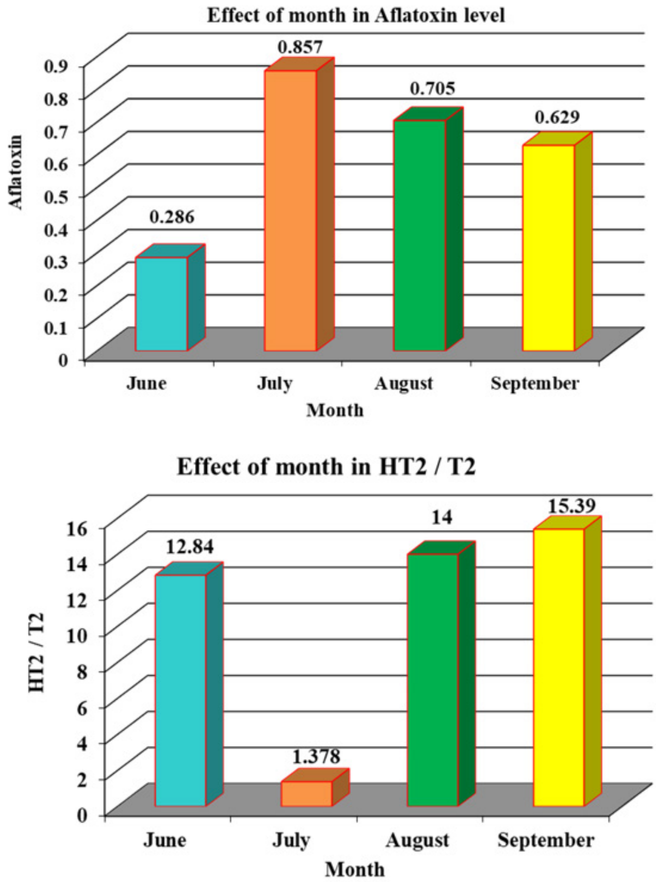

Figure 2: *Monthly variation in T-2/HT-2 toxin (upper panel) and aflatoxin (lower panel) concentrations in imported fish feeds from June to September, showing the lowest HT-2/T-2 level in July and the highest aflatoxin levels in July and August.*

Regarding ochratoxin A, the results of the quality control laboratory (Figure 3) showed that the highest mean level was recorded in July, whereas the lowest level was observed in September. July in Iraq is characterized by relatively high ambient temperatures, which, together with suboptimal storage conditions, can promote the growth of ochratoxin A–producing fungi in stored commodities (Mousa *et al*., 2016; Ali *et al*., 2017; Pietsch, 2020). This pattern is similar to the one observed for aflatoxins, where warm conditions and inadequate storage may favor fungal development and toxin formation.

To minimize this risk, mycotoxin testing is routinely performed on imported feed samples before they are used in fish farming, and appropriate storage conditions such as controlled humidity, adequate ventilation, and good warehouse management are recommended to limit fungal growth and ochratoxin A production (Ali *et al*., 2017). However, Abdulhameed *et al*. (2023) further emphasized that mycotoxins represent a global public-health concern, and that residues in animal feeds are critical within food-safety frameworks that call for strict adherence to regulations governing the feed–food chain in commercial markets and feed reception centers.

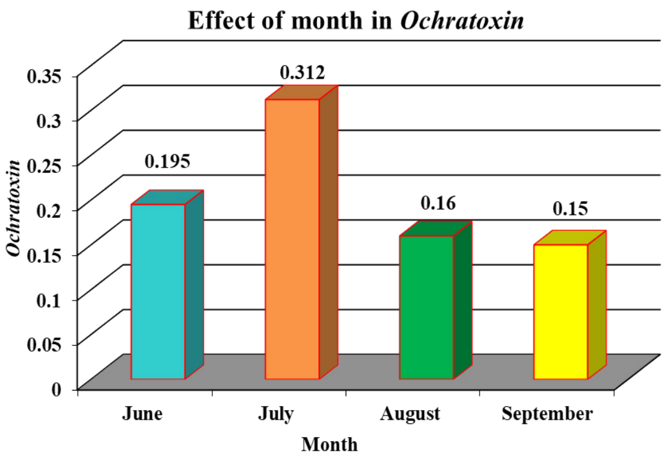

Figure 3: *Monthly variation in ochratoxin A concentrations in imported fish feeds from June to September, showing the highest level in July (0.312 ppb) and the lowest levels in August and September (0.160 and 0.150 ppb), all remaining well below the permissible limit of 5 ppb.*

Given the impact of global climate change over the past years, Iraq has been significantly affected by heat waves and reduced rainfall, leading to drought and a consequent decline in agricultural activity and livestock numbers. The country has become heavily dependent on international trade to supply consumers and farmers with essential feed ingredients (Abdulhameed *et al*., 2023).

Conclusion

This study demonstrated that imported fish feeds entering Iraq through the Al-Shaib border crossing contained detectable but very low levels of aflatoxins, T-2/HT-2 toxin, and ochratoxin A, with all values remaining well below the national permissible limits during the monitoring period. The generally low contamination suggests that current preventive measures during production, transport, and storage are effective in limiting mycotoxin exposure in imported feeds. However, the observed seasonal fluctuations, together with Iraq's growing dependence on imported feed ingredients under climate-induced pressure on local agriculture, underline the need for continuous mycotoxin surveillance at border crossings and feed reception centers. Strengthening routine testing, enforcing good storage and handling practices, and maintaining clear regulatory criteria will be critical to safeguarding fish health, sustaining aquaculture productivity, and protecting public health along the feed–food chain.

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Novelty Statement

This study provides the first systematic, border-level surveillance data on multiple mycotoxins in imported fish feeds entering Iraq through the Al-Shaib crossing, demonstrating that aflatoxins, T2/HT2, and ochratoxin A are consistently present at trace but sub-regulatory levels and exhibit seasonal fluctuations while still complying with national limits, thereby validating the current border-control system and highlighting its critical role in safeguarding fish health and aquaculture biosecurity.

Author's Contribution

All Authors contributed equally to the manuscript.

Generative AI and AI assisted technology statement

The author states that the Grammarly tool was used to improve the vocabulary of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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