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Monitoring T-2 and HT-2 Toxin Contamination in Broiler Feed: A Four-Year Survey in Duhok Governorate, Iraq (2020–2023)

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ABSTRACT

Trichothecene mycotoxins of the type A group—specifically T-2 and HT-2 toxins—are highly toxic metabolites produced by Fusarium species and are common contaminants in poultry feed, posing risks to animal health and food safety. This study evaluated the occurrence and concentrations of these toxins in broiler feed collected from farms in Duhok Governorate, Kurdistan Region, Iraq, over a four-year period (2020–2023). A total of 278 feed samples were analyzed using a commercial enzyme-linked immunosorbent assay (ELISA). Mean toxin concentrations showed a significant downward trend, declining from 85.53 µg/kg in 2020 to 32.45 µg/kg in 2023. Despite this reduction, considerable variability was observed, with the highest concentration in 2023 reaching up to 935 µg/kg, well above the European Commission guidance value of 250 µg/kg. Although average levels across the four years (32.45–85.53 µg/kg) remained below regulatory thresholds, sporadic high contamination represents an ongoing threat to poultry health and a potential source of toxin residues in poultry products. These results underscore the need for routine feed surveillance and improved storage practices, and support the development of a national mycotoxin monitoring policy to strengthen feed safety in Iraq.

KEYWORD: T-2 toxin, HT-2 toxin, broiler feed, ELISA, mycotoxins

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INTRODUCTION

Mycotoxins are a diverse group of toxic secondary metabolites produced by filamentous fungi, notably Fusarium, Aspergillus, and Penicillium species, which contaminate agricultural commodities including cereals commonly used in poultry feed [1]. Among the mycotoxins of concern, the trichothecenes particularly Type A compounds such as T-2 toxin and its metabolite HT-2 toxin are of major significance due to their potent cytotoxic, immunosuppressive, and genotoxic effects [2,3]. T-2 and HT-2 toxins are primarily

produced by Fusarium sporotrichioides and Fusarium poae, fungi that flourish in temperate regions and often contaminate grains such as maize, wheat, barley, and oats during pre- and post-harvest periods [4,5]. The occurrence and severity of contamination are influenced by multiple factors including climatic conditions, crop type, harvesting time, and storage practices [6,7].

In broiler chickens, ingestion of T-2/HT-2-contaminated feed has been associated with a wide range of clinical and subclinical effects such as decreased feed intake, reduced body weight gain, oral lesions, intestinal hemorrhage, immune dysfunction, and increased mortality rates [8,9]. These toxins inhibit DNA, RNA, and protein synthesis by binding to the peptidyl transferase site of the 60S ribosomal subunit, causing oxidative stress, apoptosis, and inflammation in rapidly proliferating tissues [10,11]. Additionally, they compromise intestinal barrier function, alter gut microbiota, and increase susceptibility to enteric and systemic infections, thereby reducing overall production efficiency and welfare in poultry operations [12–14].

Chronic exposure to low levels of T-2 and HT-2 toxins in poultry feed is particularly concerning due to the potential for tissue residue accumulation in edible products, thus posing a food safety risk for humans [15]. The carryover of these toxins or their metabolites into meat, liver, and eggs may contribute to chronic health effects in consumers and calls for strict regulatory surveillance [16,17]. Consequently, various national and international regulatory agencies, including the European Food Safety Authority (EFSA), have issued guidance levels and recommended maximum concentrations of these toxins in animal feed [18]. However, global regulatory limits vary significantly, and routine monitoring is not consistently enforced in many regions, particularly in developing countries [19].

To evaluate contamination levels and enforce safety limits, reliable detection methods are essential. While chromatographic techniques such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) offer high accuracy, they are time-consuming, expensive, and require specialized instrumentation [20]. Enzyme-linked immunosorbent assay (ELISA) has become a widely used alternative due to its high throughput, affordability, and suitability for field-based or large-scale screening programs [21]. ELISA kits targeting T-2/HT-2 toxins are commercially available and have been successfully validated in various feed matrices, though they may exhibit some degree of cross-reactivity with structurally similar trichothecenes [22].

In regions where cereal-based poultry feed is commonly used and climatic conditions favor fungal growth; the risk of T-2/HT-2 contamination is considerably elevated [23]. Thus, region-specific surveillance data are critical for understanding the scale of contamination and informing control strategies. This study aimed to determine the occurrence and year-to-year variation of the sum of T-2 and HT-2 toxins in commercial broiler feed in Duhok Governorate (2020–2023) to inform local feed-safety surveillance and risk-mitigation strategies.

MATERIALS AND METHODS

Study Area and Sample Collection

A total of 278 commercial broiler starter feed samples were collected from various poultry farms and feed mills across the Duhok Governorate of the Kurdistan Region of Iraq. Samples were collected across all four seasons, with a higher proportion obtained in summer and autumn, when elevated temperature and

humidity favor fungal proliferation, in order to evaluate the occurrence and concentration of T-2 and HT-2 toxins. The samples were collected from five major districts: Zakho, Semel, Amedi, Bardarash, and Duhok city center.

The annual distribution of the collected samples was as follows: 48 samples in 2020, 85 samples in 2021, 49 samples in 2022, and 96 samples in 2023. Samples were obtained using a sterile probe to ensure a representative subsample from multiple points within a feed bag or storage bin. Each sample (approximately 500 g) was placed in a sterile, labeled plastic bag, transported to the laboratory in a cool box, and stored at -20 °C until analysis.

Reagents and Materials

The T-2/HT-2 Toxin ELISA Kit (Veratox®, Neogen Corporation, Lansing, MI, USA) was used for the quantitative analysis. The kit components included antibody-coated microwells, T-2/HT-2 toxin standards (0, 25, 50, 100, and 200 ppb), enzyme conjugate, substrate solution, and stop solution. All reagents were brought to room temperature (20-25 °C) and mixed gently before use. Distilled water and analytical-grade methanol were used for the preparation of sample extracts.

Sample Preparation and Extraction

The frozen feed samples were thoroughly ground to a homogeneous consistency using a laboratory mill. Subsequently, 5 grams of each ground sample were weighed into a 50 mL centrifuge tube. Then, 25 mL of 70% methanol (v/v) in water was added as the extraction solvent. The mixture was vigorously shaken on a mechanical shaker for 3 minutes and then allowed to settle for 2 minutes. The extract was filtered through Whatman No. 1 filter paper. The filtrate was collected and diluted 1:5 with distilled water to minimize matrix interference and ensure the analyte concentration fell within the kit's standard curve range. This diluted extract was used directly in the ELISA procedure.

ELISA Procedure

The quantitative ELISA was performed strictly according to the manufacturer's instructions. Briefly, 100 µL of each prepared standard, control, and diluted sample extract were pipetted into separate antibody-coated microwells. The plate was gently mixed and incubated at room temperature for 10 minutes. The liquid was then decanted, and the wells were washed three times with distilled water provided in the kit. After the final wash, any residual water was removed by tapping the inverted plate on absorbent paper.

Next, 100 µL of the enzyme conjugate was added to each well, followed by a 10-minute incubation at room temperature. The wells were again decanted and washed three times as described previously. Then, 100 µL of substrate solution was added to each well and incubated for 10 minutes at room temperature in the dark. The enzymatic reaction was stopped by adding 100 µL of stop solution, which changed the color from blue to yellow. The optical density (OD) of each well was measured at 650 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA) within 15 minutes of adding the stop solution.

Data and Statistical Analysis

A standard curve was generated by plotting the mean OD values of the standards against their known concentrations (0 to 200 ppb) using a four-parameter logistic (4-PL) curve fit. The concentration of T-2/HT-2 toxins in each unknown sample was interpolated automatically from this standard curve using the Gen5 software (BioTek Instruments). All results were expressed in parts per billion (ppb) or micrograms per kilogram (µg/kg),

accounting for the initial sample weight and the dilution factor applied during extraction.

Statistical analysis was carried out using Minitab version 19 software, in accordance with the guidelines described by Montgomery [24]. A one-way Analysis of Variance (ANOVA) was employed to evaluate differences in mean concentrations of T-2/HT-2 toxins among the years of sample collection (2020, 2021, 2022, and 2023). The level of statistical significance was established at $p < 0.05$. Descriptive statistics were used to summarize the levels of T-2/HT-2 toxin contamination in the collected feed samples.

RESULTS:

A total of 278 broiler feed samples collected between 2020 and 2023 were analyzed for T-2/HT-2 toxin contamination. Descriptive statistics for each sampling year are presented in Table 1. The results demonstrated inter-annual variation in toxin concentrations, with mean values ranging from 32.45 µg/kg in 2023 to 85.53 µg/kg in 2020 Fig. 1.

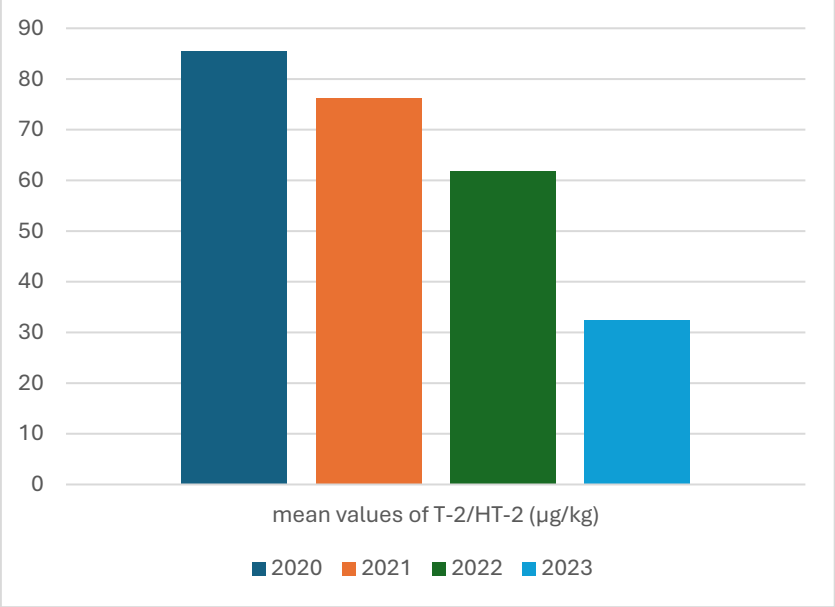

Fig 1. Trends in Mean T-2/HT-2 Toxin Concentrations in Broiler Feed Samples from Duhok Governorate (2020–2023)

In 2020, toxin concentrations ranged between 11.80 and 177.10 µg/kg (mean = 85.53 µg/kg, SD = 31.50), while in 2021, a slightly lower mean value of 76.30 µg/kg was observed, with concentrations varying from 0.00 to 164.20 µg/kg. A further decrease was recorded in 2022 (mean = 61.80 µg/kg, range = 33.00–140.00 µg/kg). Interestingly, the lowest mean concentration was reported in 2023 (32.45 µg/kg), although this year exhibited the widest variation (range = 6.30–935.00 µg/kg), reflected by the highest standard deviation (93.91).

Table 1. Descriptive statistics of T-2/HT-2 toxin concentrations (µg/kg) in broiler feed samples (2020–2023).

Year	N	Mean (µg/kg)	SE Mean	StDev	Minimum	Median	Maximum	Range
2020	48	85.53	4.55	31.50	11.80	81.10	177.10	165.30
2021	85	76.30	2.34	21.58	0.00	74.80	164.20	164.20
2022	49	61.80	2.50	17.53	33.00	62.20	140.00	107.00
2023	96	32.45	9.58	93.91	6.30	20.90	935.00	928.70

A one-way Analysis of Variance (ANOVA) was performed to assess the differences in mean toxin concentrations across the four years. The results, presented in Table 2, revealed significant differences between years ($F = 12.35$, $p < 0.001$). These findings indicate a statistically significant downward trend in mean T-2/HT-2 toxin levels from 2020 to 2023, albeit with substantial variation in the most recent year.

Table 2. The differences in mean toxin concentrations across the four years

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	3	126,852	42,284	12.35	0.000
Error	274	938,259	3,424		
Total	277	1,065,111			

The findings indicate a general decline in T-2/HT-2 toxin contamination over the four-year period; however, isolated instances of elevated concentrations were still observed, especially in 2023.

Comparison with Regulatory Limits

The detected levels of T-2/HT-2 toxins in broiler feed samples from Duhok Governorate were compared with internationally recognized regulatory guidelines. According to the European Commission (EC) Recommendation 2013/165/EU, the guidance value for the sum of T-2 and HT-2 toxins in poultry feed is 250 µg/kg [25]. Across the four years of sampling, the mean concentrations in all years (ranging from 32.45 to 85.53 µg/kg) remained below this recommended limit.

However, it is noteworthy that in 2023,

the maximum recorded value reached 935.00 µg/kg, substantially exceeding the EU guidance level.

DISCUSSION

The present study provides important insights into the occurrence and temporal variation of T-2 and HT-2 toxin contamination in broiler feed samples from Duhok Governorate between 2020 and 2023. The overall findings demonstrated a significant decline in mean toxin concentrations over the four-year study period, from 85.53 µg/kg in 2020 to 32.45 µg/kg in 2023, although considerable inter-annual variation was observed. This downward trend may reflect improved awareness of mycotoxin risks, better storage and handling practices, or differences in climatic conditions across years [26]. Similar temporal fluctuations in trichothecene contamination have been reported in Europe and Asia, emphasizing the influence of environmental factors such as rainfall, humidity, and temperature on Fusarium proliferation and toxin production [27,28].

Despite the general decline in average concentrations, isolated cases of high contamination were recorded, most notably in 2023 when one sample exceeded 935 µg/kg. This finding is consistent with the sporadic nature of mycotoxin contamination, in which most samples fall within safe levels but occasional "hot spots" present significant health risks [29]. The maximum level detected in this study substantially exceeded the European Commission (EC) guidance value of 250 µg/kg for the sum of T-2 and HT-2 in poultry feed [25]. Such exceedances are particularly concerning because even short-term exposure to high concentrations of T-2/HT-2 toxins can result in acute toxicity in broilers, including feed refusal, oral lesions, intestinal damage, and immunosuppression [30].

The mean toxin levels observed in this study (32.45–85.53 µg/kg) were below the EC guidance values but are still of biological relevance. Previous experimental studies have demonstrated that subclinical concentrations of T-2 toxin (<100 µg/kg) can impair feed conversion efficiency, alter gut microbiota, and suppress immune responses in broilers, thereby predisposing them to infectious diseases [31]. Chronic exposure, even at levels lower than regulatory thresholds, has been shown to reduce vaccine efficacy and overall flock performance [32]. Therefore, while regulatory compliance was generally maintained, the potential for long-term production losses and food safety concerns cannot be overlooked.

The seasonal distribution of samples revealed that most were collected during summer and autumn, seasons known to favor fungal growth and mycotoxin accumulation due to warm and humid conditions [33].

The use of ELISA for toxin quantification in this study provided a practical and cost-effective means of screening a large number of samples. ELISA-based methods have been widely employed in surveillance programs due to their rapidity and high throughput, although they may be prone to cross-reactivity with structurally similar trichothecenes [34]. Nonetheless, the results obtained here align with previous reports that validated the reliability of ELISA for T-2/HT-2 screening in feed matrices [22]. For future studies, confirmation of positive samples using chromatographic methods such as LC-MS/MS would provide additional accuracy and allow for multi-mycotoxin profiling [23].

The detection of sporadic high contamination in 2023 underscores the need for continuous monitoring and proactive mitigation strategies. Practical measures such as good agricultural practices (GAP), proper drying and storage of cereals, and the inclusion

of mycotoxin binders in feed formulations can significantly reduce exposure risks [35]. Furthermore, climate change and shifting weather patterns may alter the epidemiology of Fusarium species in the region, potentially leading to unpredictable contamination events [36].

Overall, the study highlights the importance of sustained surveillance of T-2 and HT-2 toxins in poultry feed in Iraq. While mean levels were below international guidance limits, the occurrence of extreme contamination events represents a serious hazard to poultry health and food safety. These findings contribute to the growing body of evidence from the Middle East, where region-specific monitoring is essential to safeguard poultry production and protect consumer health.

CONCLUSION

This four-year study confirms widespread T-2/HT-2 toxin contamination in broiler feed in Duhok Governorate, with a significant decline in mean concentrations observed over time. Nevertheless, certain samples exceeded international safety limits, highlighting the continued risk to poultry health and food safety. These findings emphasize the importance of ongoing monitoring, improved storage conditions, and the implementation of preventive measures to minimize contamination and protect both animal production and consumer health.

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