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A Survey Study on the Contamination of Broiler Feed with Ochratoxin A in Duhok Governorate, Iraq

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Abstract

Ochratoxin A (OTA) contamination in broiler chicken feed poses significant risks to animal health and food safety. This study aimed to assess OTA contamination levels in broiler feed samples collected from broiler farms in Duhok Governorate, Kurdistan Region, Iraq, over four years (2020–2023). A total of 292 feed samples were analyzed using an enzyme-linked immunosorbent assay (ELISA) to quantify OTA concentrations. The results revealed a high prevalence of OTA contamination across all years, with 86.8%, 93.8%, 100%, and 96.9% of samples testing positive in 2020, 2021, 2022, and 2023, respectively. The mean OTA concentration showed a declining trend, from 2.615 µg/kg in 2020 to 0.8396 µg/kg in 2023. Statistical analysis using one-way ANOVA confirmed a significant reduction in OTA levels over the years ($p < 0.05$), indicating improvements in feed safety management. However, variability in OTA concentrations remained high, emphasizing the need for stringent monitoring and preventive measures. The persistence of OTA in poultry feed highlights potential risks for animal health and its possible transfer to poultry products, raising public health concerns. These findings highlight the necessity of continuous surveillance, improved storage practices, and effective detoxification strategies to mitigate OTA contamination in broiler feed. Future studies should employ HPLC for more accurate OTA quantification, as ELISA, while efficient, may cross-react with other metabolites.

Keywords; Ochratoxin A, Broiler feed, Mycotoxin, ELISA.

Introduction

Ochratoxin A (OTA) is a mycotoxin produced by *Aspergillus* and *Penicillium* species, commonly found in agricultural commodities such as cereals, which are primary components of poultry feed (1, 2) These fungi proliferate under specific environmental conditions, particularly high humidity and elevated temperatures, which often occur due to improper storage of feed ingredients (3). OTA contamination in broiler feed is a major concern because of its harmful effects on poultry health, including kidney and liver toxicity, as well as immunosuppression. These adverse effects can result in poor growth performance, higher mortality rates, and considerable economic losses in the poultry sector (4-6). Additionally, OTA has been found to disrupt nutrient absorption and metabolism, ultimately reducing feed efficiency and weight gain in broiler chickens (7).

The stability of OTA during feed processing and storage further exacerbates its persistence, making it a critical challenge for food safety and animal health (8, 9). Unlike some mycotoxins that degrade under high temperatures, OTA is relatively heat-stable, allowing it to remain intact even after feed processing (10). This persistence increases the likelihood of chronic exposure in broilers, which can result in cumulative toxic effects over time (11). Recent research has also indicated the potential transfer of OTA from contaminated feed to poultry-derived products, such as meat and eggs, raising concerns about its impact on human health due to its carcinogenic, teratogenic, and immunotoxic properties (12). The presence of OTA in the food chain underscores the need for stringent monitoring and control measures to mitigate its impact on both animal and human health (13).

In addition to its direct health effects, OTA contamination in broiler feed has broader implications for food security and sustainability. As global demand for poultry products continues to rise, ensuring the safety and quality of feed ingredients is essential for maintaining productivity and meeting consumer expectations (14). Several approaches have been investigated to mitigate OTA contamination, including enhanced storage conditions, the use of mycotoxin binders, and biological detoxification methods (15). However, the effectiveness of these strategies can vary depending on the level of contamination and the specific conditions of feed production and storage (16).

Understanding the mechanisms of OTA toxicity, its prevalence in feed ingredients, and the factors influencing its production is crucial for developing effective mitigation strategies (17). Furthermore, advancements in analytical techniques, such as high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assays (ELISA), have improved the detection and quantification of OTA in feed, enabling more accurate risk assessments (18). Despite these advancements, challenges remain in fully understanding the long-term effects of low-level OTA exposure and its interactions with other mycotoxins commonly found in poultry feed (19). The study aimed to assess the prevalence, temporal trends, and variability of ochratoxin A (OTA) contamination in broiler chicken feed samples collected from Duhok Governorate, Kurdistan Region, Iraq.

Materials and Methods

Sample Collection

A total of 292 broiler feed samples were collected between January 2020 and December 2023. Sampling was distributed across all four seasons, with most samples collected in summer and autumn, which are known to favor fungal growth due to higher humidity and temperature. Samples were collected from various districts in Duhok Governorate, including Zakho, Semel, Amedi, Bardarash, and Duhok city center. The samples were distributed as follows: 53 samples in 2020, 96 samples in 2021, 47 samples in 2022, and 96 samples in 2023. Feed samples were collected directly from feed storage units or feeders using sterile sampling tools to avoid cross-contamination. Each sample weighed approximately 500 grams and was stored in clean, airtight plastic bags labeled with the collection date, location, and flock identification number. Samples were transported to the laboratory under refrigeration (4°C) and stored at -20°C until analysis to prevent degradation of mycotoxins.

Sample Preparation

Prior to analysis, feed samples were homogenized using a laboratory grinder to ensure uniformity. A representative subsample of 50 grams was taken from each homogenized sample and finely ground to pass through a 1-mm sieve. For extraction, 5 grams of the ground sample were weighed and mixed with 25 mL of 70% methanol (v/v) in a 50 mL centrifuge tube. The mixture was vigorously shaken for 10 minutes using a mechanical shaker to ensure complete extraction of OTA. The extract was then centrifuged at 4000 rpm for 10 minutes to separate the solid particles. The supernatant was collected and filtered through Whatman No. 1 filter paper to remove any remaining particulate matter. The filtrate was diluted 1:5 with distilled water to reduce the methanol concentration, as recommended by the ELISA kit protocol (20).

Ochratoxin A Detection by ELISA

The quantitative detection of OTA in the feed samples was performed using a competitive enzyme-linked immunosorbent assay (ELISA) kit provided by NEOGEN (Veratox® for Ochratoxin). The assay was conducted according to the manufacturer's instructions. Briefly, 100 µL of each prepared sample extract or standard solution was added to the antibody-coated microplate wells in duplicate. The plate was incubated at room temperature for 15 minutes to allow the OTA in the samples to compete with the enzyme-conjugated OTA for binding sites on the antibodies. After incubation, the wells were washed five times with a washing buffer to remove unbound materials. Subsequently, 100 µL of substrate solution was added to each well, and the plate was incubated for an additional 10 minutes at room temperature. The enzymatic reaction was stopped by adding 100 µL of stop solution, and the absorbance was measured at 650 nm using a microplate reader.

The concentration of OTA in each sample was determined by comparing the absorbance values to a standard curve generated using known concentrations of OTA provided in the kit. The detection limit of the assay was 1 ppb (part per billion), and the quantification range was 2–50 ppb. Samples with OTA concentrations above the upper limit of the standard curve were further diluted and reanalyzed to ensure accurate quantification.

Statistical Analysis

Statistical analysis was performed using Minitab 2019 software, following the principles outlined by Montgomery (21). A one-way Analysis of Variance (ANOVA) was conducted to compare the mean OTA concentrations across the different years of sample collection (2020, 2021, 2022, and 2023). The significance level was set at $p < 0.05$. Descriptive statistics were calculated to summarize the OTA contamination levels in the feed samples. A Fisher's Exact Test was applied to compare prevalence across the years, and significance has been reported accordingly.

Results

Prevalence of OTA Contamination

The prevalence of ochratoxin A (OTA) contamination in broiler chicken feed samples collected from 2020 to 2023 is summarized below:

2020: Out of 53 samples, 46 were positive for OTA (86.8% prevalence).

2021: Out of 96 samples, 90 were positive for OTA (93.8% prevalence).

2022: All 47 samples were positive for OTA (100% prevalence).

2023: Out of 96 samples, 93 were positive for OTA (96.9% prevalence).

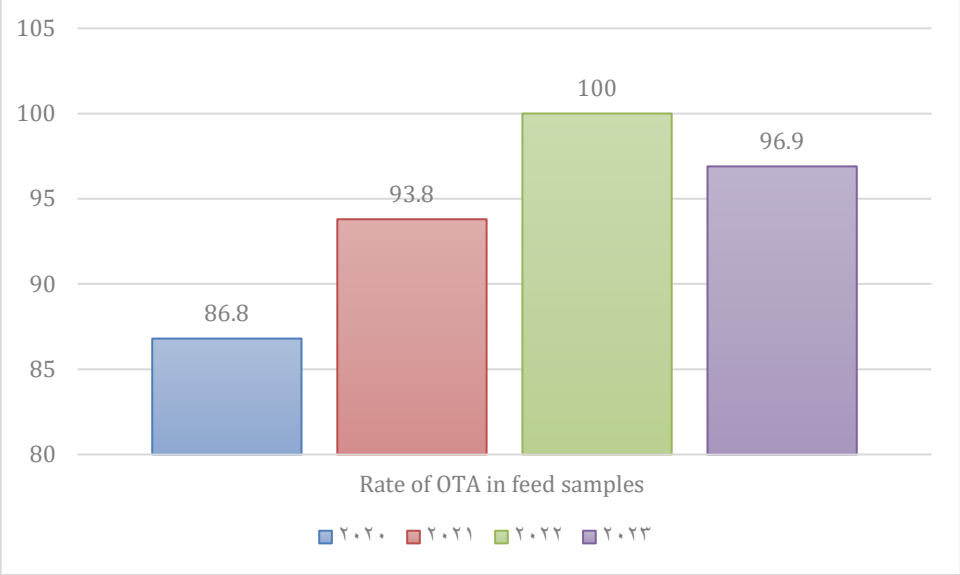

Figure 1: The distribution of OTA-positive broiler feed samples across the study years Descriptive Statistics of Ochratoxin Levels in Broiler Feed Samples (2020–2023).

Table 1 presents the descriptive statistics for ochratoxin levels across the four years. The

the highest mean level recorded in 2020 (2.615 µg/kg) and the lowest in 2023 (0.8396 µg/kg). The standard deviation (StDev) varied across years, indicating fluctuations in ochratoxin contamination levels.

mean ochratoxin concentration showed a declining trend from 2020 to 2023, with

The coefficient of variation (CoefVar) was highest in 2022 (163.63%), indicating greater variability in ochratoxin levels, while it was lowest in 2023 (69.78%), reflecting more stable contamination levels in that year. The maximum recorded ochratoxin concentration was 18.1 µg/kg in 2021, whereas the lowest

median value was observed in 2023 **(**0.7 µg/kg**),** confirming a significant reduction in contamination levels.

Comparison with Regulatory Limits

The OTA concentrations in this study were compared to the European Union maximum regulatory limit (5 µg/kg for poultry feed). While most samples were below this limit, some exceeded it, particularly in 2021 (max: 18.1 µg/kg), indicating periods of high toxicity risk.

Analysis of Variance (ANOVA) Results

A one-way ANOVA was performed to determine if there were significant differences in ochratoxin levels across the four years (2020–2023). The results are shown in Table 2.

Table 1: Descriptive Statistics and Significance of Ochratoxin Levels in Broiler Feed Samples (2020–2023).

Year	N	Mean (µg/kg)	StDev	CoefVar (%)	Min	Median	Max	Significant Difference
2020	53	2.615	2.795	106.87	0.0	2.500	11.9	a
2021	96	2.141	2.521	117.76	0.0	1.050	18.1	a
2022	47	1.547	2.531	163.63	0.1	0.400	13.7	b
2023	96	0.8396	0.5859	69.78	0.0	0.700	2.7	c

Table 2: ANOVA Results for Ochratoxin Levels

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor (Year)	3	135.2	45.063	9.71	0.000
Error	288	1337.1	4.643		
Total	291	1472.3			

The ANOVA results indicate that there is a statistically significant difference (p-value = 0.000) in ochratoxin levels among the years. The F-value of 9.71 suggests that the differences between the years are not due to random variation but rather reflect actual changes in ochratoxin contamination over time.

Discussion

This study provides an extensive evaluation of ochratoxin A (OTA) contamination in broiler chicken feed samples collected from Duhok Governorate, Kurdistan Region, Iraq, over a span of four years. Our findings reveal a high prevalence of OTA contamination, with a notable decline in mean OTA concentrations from 2020 to 2023. This declining trend suggests that improvements in feed storage and handling practices may have contributed to the reduction in OTA levels.

The prevalence of OTA contamination observed in this study aligns with findings from other countries. For instance, studies conducted in Romania and Pakistan reported significant levels of OTA contamination in poultry feed, highlighting the health implications for poultry and potential risks to human health through the

consumption of contaminated poultry products (22,23).

The mean OTA concentrations in broiler feed samples in this study ranged from 2.615 µg/kg in 2020 to 0.8396 µg/kg in 2023. These values are comparable to those reported in other studies, such as the one conducted in Pakistan where OTA concentrations ranged from 0.5 to 3.5 µg/kg (24). The declining trend in OTA levels observed in our study is consistent with findings from other researchers who have reported similar reductions in OTA contamination over time due to improved feed management practices (25,26). The statistical analysis in this study revealed significant differences in OTA levels across the four years, with the highest variability observed in 2022. This variability may be attributed to fluctuations in environmental conditions, such as temperature and humidity, which can influence the growth of OTA-producing fungi. The implementation of stringent feed storage and handling protocols is essential to minimize OTA contamination and ensure the safety of poultry feed (27-29). The findings of this study underscore the importance of continuous monitoring and management of OTA contamination in poultry feed. The observed decline in OTA levels over the four-year period is encouraging, but ongoing efforts are needed to maintain and further reduce OTA contamination.

Conclusion

This four-year study confirms widespread OTA contamination in broiler feed in Duhok Governorate, with a significant reduction in mean concentrations over time. Some levels exceeded international safety limits, emphasizing the importance of enhanced monitoring, better storage, and advanced detection techniques like HPLC. Continuous surveillance is vital to safeguard poultry health and food safety.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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دراسة مسحية حول تلوث أعلاف الدجاج اللاحم بالأوكراتوكسين A في محافظة دهوك، العراق

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الخلاصة

يشكل تلوث علف دجاج اللحم بالأوكراتوكسين A (OTA) مخاطر كبيرة على صحة الحيوان وسلامة الغذاء. هدفت هذه الدراسة إلى تقييم مستويات تلوث OTA في عينات أعلاف دجاج اللحم التي تم جمعها من مزارع الدواجن في محافظة دهوك، إقليم كردستان، العراق، على مدى أربع سنوات (2020-2023). تم تحليل ما مجموعه 292 عينة علف باستخدام اختبار المقايسة المناعية المرتبط بالإنزيم (ELISA) لقياس تركيزات OTA. كشفت النتائج عن انتشار مرتفع لتلوث OTA عبر جميع السنوات، حيث ثبتت إيجابية العينات بنسبة 86.8% في عام 2020، و93.8% في عام 2021، و100% في عام 2022، و96.9% في عام 2023. وأظهرت متوسطات تركيزات OTA اتجاهًا تنازليًا، حيث انخفضت من 2.615 ميكروغرام/كغم في عام 2020 إلى 0.8396 ميكروغرام/كغم في عام 2023. أكدت التحليلات الإحصائية باستخدام اختبار ANOVA أحادي الاتجاه انخفاضًا معنويًا في مستويات OTA على مدار السنوات ($p < 0.05$)، مما يشير إلى تحسن في إدارة سلامة الأعلاف. ومع ذلك، ظلت تباينات تركيز OTA مرتفعة، مما يؤكد الحاجة إلى مراقبة صارمة وإجراءات وقائية فعالة. يشير استمرار وجود OTA في أعلاف الدواجن إلى مخاطر محتملة على صحة الحيوان وإمكانية انتقاله إلى منتجات الدواجن، مما يثير مخاوف تتعلق بالصحة العامة. تؤكد هذه النتائج ضرورة استمرار المراقبة، وتحسين ممارسات التخزين، واعتماد استراتيجيات فعالة لإزالة السموم للحد من تلوث OTA في أعلاف دجاج اللحم. يُفضَّل أن تعتمد الدراسات المستقبلية على استخدام تقنية الكروماتوغرافيا السائلة عالية الأداء (HPLC) للحصول على قياسات أكثر دقة لتركيز الأوكراتوكسين A، وذلك لأن تقنية الإليزا، على الرغم من كفاءتها، قد تُظهر تفاعلات متقاطعة مع نواتج أيضية أخرى.

الكلمات المفتاحية: الأوكراتوكسين A ، علف دجاج التسمين ، السموم الفطرية، ايلايزا.