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Trends in Aflatoxin B1 Contamination in Broiler Feed: A Three-Year Study from Duhok Governorate

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Abstract This study assesses the levels of aflatoxin B1 contamination in broiler feed samples that were taken from farms in the Duhok Governorate between January 2020 and August 2022. An ELISA technique used to evaluate 213 feed samples in total. The data indicated a significant increase in aflatoxin B1 contamination, with 22 samples (10.32%) testing negative and 191 samples (89.67%) testing positive. Every year from 2020 to 2022, the proportion of samples that tested positive for contamination increased, going from 76.36% in 2020 to 91.50% in 2021 and 100% in 2022. The concentration of aflatoxin B1 also showed an increased trend, with mean values of 5.351 ppb in 2020 and 6.338 ppb in 2022. Descriptive statistics indicated considerable variability in aflatoxin levels, with the highest mean observed in 2022. ANOVA results confirmed statistically significant differences between years (F-value = 4.36, p-value = 0.014), highlighting a concerning escalation in contamination levels. This rise emphasizes the need for improved management and monitoring techniques to reduce aflatoxin exposure. The results are consistent with prior research showing elevated levels of contamination brought on by environmental factors and feed management techniques. The research highlights the significance of enforcing more stringent quality control protocols to guarantee feed safety and safeguard the well-being of chickens.

Keywords: Aflatoxin, Broiler, ELISA, Feed

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Introduction

Animal health and agricultural commodities are seriously threatened by aflatoxin B1 (AFB1), one of the most poisonous and carcinogenic mycotoxins, especially in the poultry industry (1). The molds Aspergillus flavus and *Aspergillus parasiticus*, which frequently contaminate crops are the source of AFB1, which was discovered in the 1960s (2,3). AFB1's widespread presence in poultry feed has caused major concerns because of its detrimental effects on the health and productivity of broiler chickens (4,5)

Warm, humid environments are favorable to the molds that produce AFB1, which can contaminate a variety of feed ingredients (6-8). Because they are susceptible to fungal infection during growth and storage, corn, peanuts, and cottonseed are especially vulnerable to aflatoxin contamination (9). Contamination in broiler feed might come from contaminated source materials or from the production process itself (10).

The liver is the primary site of AFB1 metabolism. There, it undergoes bioactivation to generate extremely reactive intermediates that attach to DNA and proteins in cells (11, 12). This metabolic stimulation causes oxidative stress, DNA damage, and eventually cancer. AFB1 causes hepatocellular necrosis and increases the risk of liver cancer by interfering with normal cellular processes in liver cells (13-15).

AFB1 has a sever negative impact on broiler health. Acute exposure can result in poor feed conversion ratios, decreased growth rates, and damage to the liver (16). Prolonged exposure is linked to long-term health problems such as immune system suppression, an increased risk of infections, and reduced organ function (17). Contamination of broiler feed with AFB1 carries considerable public health risks, given that residues of this mycotoxin can be transferred to

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poultry products intended for human consumption (18).

The financial consequences of AFB1 contamination are significant, affecting both the poultry industry and consumers. Reduced growth rates and increased veterinary costs translate into financial losses for producers, while the potential for aflatoxin residues in poultry products poses risks to human health and can lead to trade limitations (17). To reduce these dangers, regulatory agencies such as the FDA and EFSA, have set maximum allowed limits for AFB1 in feed; nonetheless, enforcement and compliance are still difficult issues (18).

Controlling the risks of contamination requires accurate aflatoxin B1 detection in feed. Aflatoxin levels can be measured using a variety of analytical techniques, such as mass spectrometry, enzyme-linked immunosorbent assays (ELISA), and high-performance liquid chromatography (HPLC). AFB1 contamination in feed must be detected early and prevented with the use of regular monitoring and surveillance programs (10,19).

Several techniques are used to reduce the dangers connected to AFB1. Among these are biological detoxifiers that break down aflatoxins and the use of aflatoxin adsorbents, such as those based on clay (17,20). There is also a chance of less contamination if feed storage conditions are improved and excellent production methods are used (21, 22). The present study aimed to assess the prevalence and concentration of AFB1 contamination in broiler chicken feed in the Duhok governorate of Iraq.

Materials and methods

Ethical approval

The study was done according to the approval recorded under the number (CVM2020/0201UoD) in 02/01/2020 issued by the College of Veterinary Medicine, University of Duhok, Iraq.

Between January 2020 and August 2022, 213 broiler feed (n=213) samples were taken from broiler farms in the Duhok Governorate, Kurdistan Region, Iraq. The samples were collected from the same farms during the same months of each year. The distribution of the sample was as follows: In 2020, 55 samples were gathered; in 2021, 106 samples; and in 2022, 52 samples. Samples were collected from various farms in order to ensure a regionally representative analysis.

Sample Preparation

1. Homogenization: To obtain a uniform consistency, each diet sample was thoroughly mixed.
2. Sample Subdivision: Approximately 50 grams of each mixed feed sample was taken for extraction and analysis.

Extraction:

- The feed sample was ground to a fine powder.
- A subsample (5 grams) was weighed and mixed with 50 mL methanol-water (80:20 v/v).
- The mixture was shaken vigorously for 30 minutes using a mechanical shaker.
- The sample was then centrifuged at 4000 rpm for 10 minutes to separate the supernatant from the solid residue.

Filtration: Before analysis, the supernatant was filtered through a 0.45 µm syringe filter to remove any particles (23).

Aflatoxin B1 Detection

An Enzyme-Linked Immunosorbent Assay (ELISA) from Neogen Corporation was used to measure the level of aflatoxin B1 in the feed samples.

ELISA Procedure:

Reagents and samples were equilibrated to room temperature, and necessary solutions (e.g., sample diluent, wash buffer) were prepared according to the kit instructions. Feed sample extracts were diluted with the sample diluent, and AFB1 standards were prepared to create a standard curve (0–50 ppb).

For the assay, 100 µL of AFB1 standards, diluted samples, and controls were added to the microtiter plate wells and incubated for 60 minutes at room temperature. Afterward, wells were washed four times with wash buffer. Next, 100 µL of enzyme conjugate (HRP) was added to each well, followed by another 60-minute incubation, and subsequent washing. Substrate solution (TMB) was then added and incubated for 15–30 minutes in the dark. The enzymatic reaction was stopped by adding 50 µL of sulfuric acid stop solution.

Absorbance was measured at 450 nm within 15 minutes using a microplate reader, and a standard curve was generated by plotting the absorbance values of the standards against their concentrations.

Data Analysis:

- The concentration of aflatoxin B1 was calculated for each sample using the standard curve.
- The results expressed in parts per billion (ppb).
- The contamination levels compared against regulatory limits to assess compliance.

Statistical Analysis

To analyze the differences in aflatoxin B1 levels among the three years, a one-way Analysis of

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Variance (ANOVA) was performed using Minitab 2019 software. The statistical significance of differences in contamination levels among 2020, 2021, and 2022 was assessed with a significance level set at $p < 0.05$. ANOVA results were used to determine whether the observed variations in aflatoxin B1 levels across the years were statistically significant.

Results

Out of 213 broiler feed samples collected between January 2020 and August 2022 and tested for aflatoxin B1 contamination, 22 samples (10.32% of the total) were found to be negative, while 191 samples (89.67% of the total) tested positive.

The distribution of positive and negative samples by year is as follows:

In 2020, out of 55 samples, 13 were negative (23.63%) and 42 were positive (76.36%). In 2021, of the 106 samples collected, 9 were negative (8.49%) and 97 were positive (91.50%). In 2022, all 52 samples tested were positive (100%), with no negative samples figure 1.

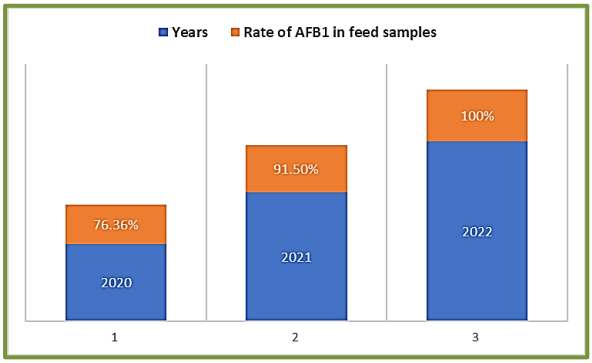

Figure 1: The distribution of positive broilers feed samples for AFB1 by year.

Descriptive Statistics

Table 1: summarizes the basic descriptive statistics of aflatoxin B1 concentrations in positive samples across the years.

Metric	2020	2021	2022
N	55	106	52
Mean	5.351	3.894	6.338
SE Mean	0.868	0.493	0.428
StDev	6.439	5.077	3.086
CoefVar (%)	120.34	130.38	48.69
Minimum	0	0	1
Q1	0.1	0.4	4.325
Median	4.1	2.05	6.2
Q3	7.3	5.15	8.825
Maximum	29.5	22.1	13.6

Analysis of Variance (ANOVA)

A one-way ANOVA was performed to determine if there were significant differences in aflatoxin B1 levels across the years. The results of the ANOVA are presented in Table 2.

Table 2: Analysis of Variance for Aflatoxin B1 Levels

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	225.8	112.88	4.36	0.014
Error	210	5431.8	25.87		
Total	212	5657.5			

The ANOVA results indicate that there are statistically significant differences in aflatoxin B1 levels between the years (F-value = 4.36, p-value = 0.014). This significant p-value suggests that the variations in aflatoxin B1 concentrations observed over the years are not due to random chance but are likely due to actual differences between the years.

Discussion

Aflatoxin is the most studied toxin since it is associated with a high rate of disease and mortality in poultry. Thus, aflatoxin contamination in different feed grains poses a significant risk to public health and harms the wellbeing of both people and animals (24). This study highlights a troubling increase in aflatoxin B1 contamination in broiler feed in the Duhok Governorate, with contamination rates rising from 76.36% in 2020 to 100% in 2022. These findings reflect a significant upward trend in contamination levels, raising serious concerns for industry stability.

The high percentage of aflatoxins in total may be the result of improper storage practices for chicken feed at the farms. Furthermore, there's a chance that additional factors like inadequate ventilation and temperature control systems contribute to a greater percentage of contamination (25).

This trend reflects an overall increase in aflatoxin contamination, consistent with observations by Lubna et al. (23), who collected 100 samples from various farms in Bangladesh and reported that 97% were contaminated with AFB1. Another study from Pakistan conducted by Naveed et al. (26) documented that 92.5% of samples tested positive for AFB1 contamination. In Brazil, Rossi et al. (27) recorded a high AFB1 contamination rate of 88.2%, attributed to favorable conditions for fungal growth. The high

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contamination levels observed in 2022, with no negative samples, suggest a serious and worsening problem, which aligns with the findings of Shephard (28), who noted that aflatoxin contamination can become widespread if not managed effectively. Conversely, a study conducted in Jordan by Alshawabkeh et al. (29) reported that only 23.07% of samples tested positive for aflatoxin, significantly lower than the results of our study; this might be due to improved storage and climatic conditions.

The significant rise in aflatoxin contamination observed in this study underscores the urgent need for improved monitoring and control measures. Effective strategies, including better storage conditions and regular testing, are crucial in mitigating aflatoxin risks (30-32). The results suggest that more stringent regulations and proactive management practices are necessary to address the escalating problem of aflatoxin contamination in broiler feed.

Conclusion

This study highlights a significant increase in aflatoxin B1 contamination in broiler feed from Duhok Governorate, with positive samples rising from 76.36% in 2020 to 100% in 2022.During the course of the investigation, there was a notable increase in the average concentration of aflatoxin B1. These patterns were verified as significant by statistical analysis (F-value = 4.36, p-value = 0.014). The aforementioned results highlight the pressing necessity for intensified monitoring and control protocols to tackle the increasing levels of contamination. To safeguard the health of chickens and guarantee feed safety, immediate improvements in feed storage and testing procedures are necessary.

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Conflicts of Interest

The authors declare there is no conflict of interest.

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