Online ISSN: 2588-3526
Journal of Wildlife and Biodiversity
Volume 9(2): 331-340 (2025) (http://www.wildlife-biodiversity.com/)
Research Article

Monitoring of aflatoxins in captive avifauna feed in summer and monsoon seasons

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Received: 15 January 2025 / Revised: 21 May 2025 / Accepted: 25 May 2025/ Published online: 25 May 2025.
How to cite: Younis, M., et al. (2025). Monitoring of aflatoxins in captive avifauna feed in summer and monsoon seasons, Journal of Wildlife and Biodiversity, 9(2), 331-340. DOI: https://doi.org/10.5281/zenodo.15520822

Abstract

Aflatoxins are the most common types of mycotoxins that are further categorized as AFB1, AFB2, AFG1, and AFG2. They are toxic metabolites and are produced by fungi, mostly *Aspergillus flavus* and *Aspergillus parasiticus*. In the present research, aflatoxins B1, B2, G1, and G2 were monitored in the feed of captive birds. A total of 40 feed samples were collected in summer and monsoon season from Lahore Zoo from cages of ten bird species such as Ostrich (*Struthio camelus*), Black Swan (*Cygnus atratus)*, Green pheasant (*Phasianus versicolor)*, Silver Pheasant (*Lophurany cthemera*), Crane Bird (*Grus virgo*), Chukar **(**Alectoris *chukar*), Red-Wattled Lapwing *(Vanellus indicus)*, Finches (*Fringillidae*), Alaxandrine parakeet (*Psitta culaeupatria),* and Turkey Bird (*Meleagris*). The Thin Layer Chromatography (TLC) method was used to detect and estimate the level of aflatoxins. Upon quantification, out of 40 samples 21 were contaminated, and of them, 17 were within the permissible range, while 4 were contaminated beyond the permissible range. The tolerant range of the limit of aflatoxins in avifauna feed daily was 10 µg/kg according to the EU (2010). AFB1 was detected in 21 samples, whereas B2, G1, and G2 were not detected in any sample. The overall percentage of contaminated feed samples was lower in summer (35%) as compared to the monsoon season (65%). Statistical analysis, such as an independent sample t-test, was done by using SPSS

version 22, and significant differences (<0.05) were noticed in aflatoxin levels in summer and monsoon season. However, there were non-significant differences that were noticed among the feed samples of different birds by ANOVA. It was concluded that the storage condition of feed samples was better in Lahore Zoo. However, in the monsoon season, there is a need of special care by the feed regulating authorities of the Zoo so that the spread of aflatoxins can be reduced.
Keywords: Aflatoxins, Thin Liquid Chromatography, summer, monsoon

Introduction

Mycotoxins were detected in the 1960s and mostly produced by *Aspergillus flavus*, and *Aspergillus parasiticus* (Richard, 2007; Hussain et al., 2023). There are four major types of aflatoxin, namely B1, B2, G1, and G2 (Summia et al., 2020). These aflatoxins can be detected by using different techniques such as TLC, HPLC, and ELISA (Iqbal et al., 2014). Although nowadays biosensors and polymerase chain reaction methods are in practice (Faria et al., 2012; Majer-Baranyi et al., 2023; Szelenberger et al., 2024). The levels of aflatoxins in feed are 300 ppb for beef and poultry, 20 ppb for egg laying hens, and 3 ppb for ducks, are established by the US Food and Drug Administration in 2009 (Liu et al., 2016; Chowdhury et al., 2018).

Aflatoxicosis in birds reduces the usage of feed, which results in immunosuppression, decreased development (Fraga et al., 2007; Monson et al., 2015; Agriopoulou et al., 2020). The result of aflatoxin on homegrown cultivated avifauna species has been widely explored (Arné et al., 2021). The portions of 1250, 2500, and 5000 kg (ppb) in the food were taken in care for a considerable length of time, showing the scope of amounts in chicken that are poisonous (Marin et al., 2013).

It is reported that aflatoxins are harmful for wildlife and hepatic residues were noticed for the first time in wild birds (House sparrows and greenfinches) in Britain (Lawson et al., 2006). Marchioro et al. (2013) mentioned the negative impact of aflatoxins on broilers' growth performance and pancreas. Harmful effects of *A. flavus* and *A. parasiticus* in birds have been reported (Fouad et al., 2019). A level of 500 ppb was found toxic for the turkey, but no effects were noticed at 250 ppb (Diaz et al., 2009). The number of aflatoxin research studies in the past has been conducteddue to the financial implications of chronic exposure to the fowls' sector or to assess possible public health concerns from consuming infected eggs, milk, or meat. Though in past years, there outdated a lot of interest in the research of aflatoxicosis in free-roaming wildlife species (Hussain et al., 2010).

Irum et al. (2019) studied 204 home mix poultry layer feed samples, and the highest concentration of *A. flavus* was reported in the months of June to August as compared to

September to November. Overall, the highest rate of contamination was recorded in 132 samples out of 204, with a percentage of 64.7 %. In the current study, the level of aflatoxins was compared in captive avifauna feed samples in summer and monsoon seasons.

Material and methods

Collection of Samples

The avifauna feed samples were collected from the cages of ten species, such as Ostrich (*Struthio camelus*), Black Swan (*Cygnu satratus)*, Green pheasant (*Phasianus versicolor)*, Silver Pheasant (*Lophurany cthemera*), Crane Bird (*Grus virgo*), Chukar **(**Alectoris *chukar*), Red-Wattled Lapwing *(Vanellus indicus),* Finches (*Fringillidae*), Alaxandrine parakeet (*Psitta culaeupatria),* and Tukey Bird (*Meleagris*) were selected in summer (May to June) and monsoon season (August to September) from Lahore Zoo. The research work was carried out in the aflatoxin laboratory, food, and biotechnology research center, Pakistan Council of Scientific and Industrial Research (PCSIR), Lahore.

Preparation of Samples

A feed sample of 100 g was taken from each cage and stored in plastic zipper polythene bags. After that, 50 g of each sample was taken in a flask and further prepared for the determination and quantification of Aflatoxins by Thin Layer Chromatography. Feed samples were then properly mixed and ground to get a fine powder form for better experimental analysis. The chloroform method was selected to extract aflatoxins. The type of aflatoxins that were extracted and analyzed from the feed samples included AFB1, AFB2, AFG1, and AFG2. The extraction method was carried out by taking about 50 g of each ground feed sample in a 500 ml Erlenmeyer flask. About 25 ml of water and 150 ml of chloroform were poured into the flask. Each flask was then covered with Aluminum Foil and then shaken for 30 minutes with the help of a wrist-action shaker. After shaking, the feed sample was filtered through Whatman filter paper. About 50 ml of the Filtrate was taken in a beaker and then put on a hot plate for evaporation (Summia et al., 2020).

Development and Redeployment of TLC Plate

The dilutions were obtained in microliters for spotting purposes. With the help of a micro syringe, a spot of 25 µL of the test solution was then applied on a Layer Chromatographic Plate. Standard spots of 5 µL and 10 µL of aflatoxins AFB1, AFB2, AFG1, and AFG2 were also spotted on that plate to work as internal standards. The TLC plate was placed in a TLC tank

containing anhydrous ether until the solvent travelled halfway up. After the plate was finely developed, it was taken out and dried. The re development of plate was then done in a similar direction but with solutions ofacetone and chloroform to a ratio of 1:9. After the removal of plate from the tank the spot of test solution was properly observed under Ultra-Violet light to detect presence or the absence of aflatoxins (Summia et al., 2020).

Statistical Analysis

Statistical analysis was done by using SPSS version 22. Independent sample t-test and one-way analysis of variance (ANOVA) were performed to compare the summer and monsoon seasons and to see the differences among the feed samples.

Results

Levels of aflatoxins were detected in 40 feed samples of the selected species. All the samples were collected from Lahore Zoo belonging to two seasons, i.e., summer (May to June) and monsoon (August to September). All the samples were processed into replicates.

Samples collection in the Summer Season (May-June)

In May, the levels of aflatoxins were detected in feed samples collected from the cages of ten selected species. Four samples were found to be positive while no aflatoxins were detected in the other six feed samples (Table 1). The highest levels of aflatoxins were detected in the Alexandrine parakeet (56.71 ppb), followed by the TurkeyBird (37.52 ppb). The least quantity of aflatoxins was detected in Chukar (12.18 ppb) and Black Swans (12.02 ppb) shown in Table 1. In June, only 3 samples were found to be positive, while no aflatoxins were detected in the other 7 samples. The highest levels of aflatoxins were detected from the cages of green pheasant (12.07 ppb) as compared to Ostrich (12.04 ppb) and Silver Pheasant (11.92 ppb), as shown in Table 1.

Table 1. Level of Aflatoxins in feed samples in the summer season (May and June)

Sample Name	Scientific Name	May	June	Within PR	Beyond PR
Ostrich	*Struthio camelus*	ND	12.04 ppb	1	-
Black Swans	*Cygnu satratus*	12.02 ppb	ND	1	-
Green Pheasant	*Phasianus versicolor*	ND	12.07 ppb	1	-
Silver Pheasant	*Lophurany cthemera*	ND	11.92 ppb	1	-
Crane Bird	*Grus virgo*	ND	ND	-	-
Chukar	Alectoris *chukar*	12.18 ppb	ND	1	-
Red-wattled Lapwing	*Vanellus indicus*	ND	ND	-	-

Finches	*Fringillidae*	ND	ND	-	-
Alexandrine parakeet	*Psitta culaeupatria*	16.71 ppb	ND	1	-
Turkey Bird	*Meleagris*	37.52 ppb	ND	1	-

Samples collection in the Monsoon season (August-September)

A total of 10 feed samples were collected in mid-August. In 3 feed samples, aflatoxins were detected positively. While no aflatoxins were found in the other 7 samples positive. Whilst the level of aflatoxins wasdetected in Silver Pheasant (7.32 ppb) followed by Red-Wattled Lapwing (7.98 ppb) and Finches (7.97 ppb). On September 9, out of 10 feed samples were detected positive. The highest level of aflatoxins was detected in Ostrich (56.1ppb), Green pheasant (42.93ppb), Black Swans (30.3ppb), and Turkey Bird (24.15ppb). While the lowest level was detected in Red-Wattled Lapwing (8.01ppb), Silver Pheasant (7.65ppb), Alexandrine parakeet(7.32ppb), Chukar (3.86ppb), and Crane Bird (3.16ppb), as shown in Table 2. In August, all three (Silver Pheasant, Red-Wattled Lapwing, and Finches) Aflatoxins detected feed samples were within the permissible range. While in September, Aflatoxins were detected in 9 samples. Only one sample (Ostrich) was beyond the permissible range. While the other eight samples were within the permissible range (Table 2).

Table 2. levels of Aflatoxins in feed samples in monsoon season (August and September)

Sample Name	Scientific Name	August	Sep	Within PR	Beyond PR
Ostrich	*Struthio camelus*	ND	56.1 ppb	-	1
Black Swans	*Cygnu satratus*	ND	30.3 ppb	1	-
Green Pheasant	*Phasianus versicolor*	ND	51.93 ppb	1	1
Silver Pheasant	*Lophurany cthemera*	7.32 ppb	7.65 ppb	2	-
Crane Bird	*Grus virgo*	ND	3.16 ppb	1	-
Chukar	Alectoris *chukar*	ND	3.86 ppb	1	-
Red-wettled Lapwing	*Vanellus indicus*	7.98 ppb	8.01 ppb	2	-
Finches	*Fringillidae*	7.97 ppb	ND	1	-
Alaxandrine parakeet	*Psitta culaeupatria*	ND	7.32 ppb	1	-
Turkey Bird	*Meleagris*	ND	24.15 ppb	1	-

Analysis of contaminated Samples

Out of 40 samples, 19 samples were contaminated out of which 17 were within permissible range and 2 were beyond the permissible range. The samples beyond the permissible range were unfit for consumption and could cause serious health problems. The samples within the permissible

range can be given to birds as they are under the tolerant range (10 ug /kg) on daily basis according to EU (2010). AFB1 was detected in 19 samples whereas B2, G1 and G2 were not detected.

The percentage of 17 contaminated samples within the permissible range was 47.5%. While the remaining 2 contaminated samples, beyond the permissible range were 25%. The percentage of 21 uncontaminated samples was 52.5%.

Table 3. A detailed percentage of total contaminated and uncontaminated feed samples

Seasons	No. of uncontaminated samples	No. of contaminated samples	No. of contaminated samples within the permissible range	No. of contaminated samples beyond the permissible range
Summer	13	7	7	0
Monsoon	8	12	11	2
Total	21	19	17	2
Percentage	52.5%	47.5%	42.5%	5%

Discussion

Aflatoxins are poisonous metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Lawson et al., 2020; Hussain et al., 2023). The present study was conducted to quantify the aflatoxins (B1, B2, G1, G2) in captive avifauna feed samples. A total of 40 samples were collected from the Lahore Zoo in the summer and monsoon seasons. The samples were collected from June to September 2021. Out of 40 samples, 17 were contaminated within the permissible range, and their percentage was 42.5%. While 2 contaminated samples were beyond the permissible range and their percentage was 5%. The 21 samples out of 40 were uncontaminated, and not a single type of aflatoxin (B1, B2, G1, and G2) was detected. The uncontaminated samples percentage was 52.5% and fit for consumption. While a study conducted in Istanbul in which 102 feed samples were collected and not a single aflatoxin was detected in any sample (Korkmaz et al., 2017). A higher percentage of samples were recorded in the monsoon season compared to the summer season. While a study carried out by Hussain et al. (2023) reported a higher percentage of contaminated samples compared to our results, and the highest contaminated bird feces samples in summer compared to winter season. Abdou et al. (2017)

mentioned the highest contamination in the summer season compared to the winter season. While a study from North India showed higher contamination in monsoon and the findings of the study are like our results (Dutta and Das, 2001). The maximum value of aflatoxins detected in avifauna feed on a daily basis was 10 µg, which was very little (2,072 µg/kg) as mentioned by Pleadin in 2012.

Rashid et al. (2013) conducted a study with 96 feed samples collected from various broiler chicken farms in the district of Quetta, Pakistan. The samples were evaluated for aflatoxins using thin-layer chromatography, and 48.8 % of the samples were found to be contaminated with aflatoxin B1. Higher contamination levels were noticed compared to existing findings, where only 42.5% of samples were contaminated. Aflatoxins are fungal toxins in poultry feeds that diminish feed quality and harm bird health. Their presence highlights the need for stringent feed monitoring and control strategies (Mgbeahuruike et al., 2013). In another study, 40% of the total feed samples were found to be contaminated with aflatoxins, and a commercial ELISA kit was used for the detection of aflatoxin (Queiroz et al., 2013). A study depicted that adding one mg/kg of aflatoxin B1 in birds' diet negatively impacted the performance of broiler chicken and can damage the liver (Oliveira et al., 2015).

Mérida et al. (2020) compared different advanced methods, such as sensing platforms and fluorometry in combination with antibodies that are used for aflatoxin detection. But in the current research, the TLC method was used, which was simple and cheap as compared to fluorometry and monoclonal antibody-based immune-affinity columns. Mahfuz et al. (2020) in their review study reported that various advanced techniques, such as Enzyme-Linked Immunosorbent Assay (ELISA), Thin-Layer Chromatography (TLC), and High-Performance Liquid Chromatography (HPLC), are used to identify aflatoxins. All the methods are varied in sensitivity, specificity, and usability.

Conclusion

It was concluded that the percentage of aflatoxins in avifauna feed at Lahore Zoo was higher during the monsoon season compared to the summer season. Although 47.5% of the feed samples were found to be contaminated, the levels were within permissible limits. Only 5% of the samples exceeded these limits during the monsoon season. The study reflects a generally good standard of feed at Lahore Zoo, but it highlights the need for stricter monitoring,

particularly during the monsoon season, to ensure the provision of high-quality feed to captive avifauna.

Acknowledgement

Authors acknowledge LGU and PCSIR Lab staff.

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