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Monitoring of Aflatoxins in Broiler, Quail and Ostrich feed samples

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Abstract

Aflatoxins are types of biological toxins also known as mycotoxins and can be produced by 100 different types of fungi. The most common types of mycotoxins are AFB1, AFB2, AFG1, and AFG2. These are toxic metabolites that are produced by *Aspergillus flavus* and *Aspergillus parasiticus*. In the present study, the most common method thin layer chromatography (TLC) was used to estimate the levels of aflatoxins in broiler, quail, and ostrich feed samples. A total of 18 samples were collected from different sites of broiler, quail, and ostrich farms and local shops in Lahore. Upon quantification by TLC method, the contamination was detected in 12 samples (66.66%) out of 18 samples. B1, B2, and G1 aflatoxins were detected in different feed samples. The prevalence of the aflatoxins in overall feed samples was noticed in the following order B1>B2>G1. A less amount of contamination was noticed in broiler feed samples as compared to quail and ostrich feed samples. Results were statistically analyzed by one-way ANOVA and significant differences at p-value < 0.05 were noticed for aflatoxins in broilers with quail and ostrich. While non-significant differences at p-value> 0.05 were noticed for ostrich and quail feed samples. By independent sample T-test, a significant difference was noticed in positive feed samples of summer and winter seasons at p-value < 0.05. The most contaminated samples were found in the summer season as compared to the winter season. However, it was concluded that might be the storage conditions of feed samples need to improve and should be regulated by feed authorities so that the effects of aflatoxins can be reduced in the feed samples.

Keywords: Aflatoxins, Detoxification, Quail, broiler, Ostrich, Contamination

Introduction

Aflatoxins are types of biological toxins and a reason for acute as well as chronic compulsive disorders in farm animals (Peles et al., 2019). Different filamentous fungi give rise to mycotoxins which are considered secondary toxic metabolites (Nielsen et al., 2009; Fernández-Cruz et al., 2010; Egbuta et al., 2017). The mycotoxins are produced when these agents face stressful environments such as temperature variations and humidity (Amadi and Adeniyi, 2009; Mannaa and Kim, 2017). Most poultry feed grains contaminated with fungi are fed to different animals which results in great economic loss. In the USA, different mortality incidents have been related to acute aflatoxicosis in waterfowl due to contaminating feed (Lawson et al., 2006).

In different mycotoxins, the most pathogenic one is AFB (aflatoxin B) which is primarily formed by the Aspergillus species particularly *A. parasiticus* and *A. flavus* (Perrone and Gallo, 2017). A study elaborates on the negative effects of mycotoxin on chicken's rate of growth, feed and reproduction of these animals (Bryden, 2012). These toxins cause damage to different tissues of birds like the liver, heart, and spleen as well as the bursa of Fabricius, so enhancing the cost for poultry industries for the production of chicken. These changes in the bird tissue and size cause a great loss to the industry of poultry (Ghaffar et al., 2018; Hassanen et al., 2020; Abdelrahman et al., 2022). However, further studies are required in order to find out the sources of dietary aflatoxins as well as their pathological effects on the wild birds of Britain (Lawson et al., 2006).

Chicken is a widely used food in the world as it is a rich source of nutrients i.e. proteins, fats, minerals and vitamins (Marangoni et al., 2015; Lesnierowski and Stangierski, 2018; Saadaoui et al., 2022). The farming of chicken is practiced all over the world including our country (Ornelas-Eusebio et al., 2020). In the last few years poultry production and the market have faced different negative incidents due to aflatoxins which reduce the value of chicken (Schröder and McEachern, 2004).

Ostrich natural feed mainly consists of green grasses, seeds, small insects and berries. In pasture, the fully grown ostrich feeds on dry matter three times more than the dairy cow regardless of the total body weight percentage. Feeding cost is a fundamental concern during ostrich farming. A chick grown in optimum conditions showed an optimum growth rate and is less susceptible to different diseases. So, it is much more important to feed the young ones optimally so they can acquire the best possible slaughter weight ranges from

90 to 100 kg as soon as possible (Degen et al., 1991). Ostriches' food mainly comprises 60% of plants, 15% of fruits, 4-5% of food consists on insect eggs and other mammals which are small in size, and the remaining diet consists of salts, stones and cereal grains. Ostriches require continuous feeding rather than feeding at intervals, so a continuous diet should be made available to ostriches (Aganga et al., 2003). Aflatoxins are produced by storage fungi belonging to the genus Aspergillus during their growth on food and food components (Coulombe, 1991). A total of 18 different types of aflatoxins have been determined so far and the most important are B1, B2, G1 and G2 (Hsieh and Atkinson, 1991).

Different poultry species have different susceptibility to aflatoxins. Their susceptibility mainly depends upon age, sex and breed. The same scenario is followed in quails as different researches indicate that the Japanese quail showed much more resistance than the Bobwhite quail (Oberheu and Dabbert, 2001). The main organ which is targeted by aflatoxins in quails is the liver which causes fatty infiltration and hyperplasia in the bile duct of quails. Chronic aflatoxicosis is usually caused by aflatoxins which results due to poor performance of dietary intake. In chickens, AFB1 causes a decrease in immunity, body weight, egg production and egg weight (Tessari, 2006).

The present study aimed to detect levels of aflatoxins in the feed of broiler, quail and ostrich feed samples in the summer and winter seasons.

Materials and methods

The present study was designed on comparative analysis of aflatoxins in feed samples of broiler, quail and ostrich in two different seasons (summer and winter).

Sample Collection and Labelling

Samples of feed were collected from poultry, quail and ostrich feed in zipped polythene bags and properly labelled as shown in (Fig. 1).

Figure 1. Collection and labelling of samples

Sample Size and Collection Site

A total of eighteen feed samples were collected in the summer and winter seasons, from poultry, quail and ostrich feed samples. The bird feed samples were collected from the local feed shops of Lahore and Sheikhupura.

Processing and Preparation of Samples

Poultry feed samples were processed for the determination of aflatoxins by following the procedures of Begum et al. (1985). For the preparation of samples, 50g of ground samples were kept in 500 ml conical flask and 225 ml of chloroform and 25 ml of water were added. Flasks were placed on a wrist action shaker (Model 75) for 35 minutes and samples were filtered in beakers by filter paper. Filtrate with a 50 ml volume of chloroform was collected in beakers and placed to heavy-duty hotplate (HDHP-1A/04) for evaporation.

Spotting on Thin Layer Chromatography (TLC) Plate

The remaining sample was collected and diluted further for spotting in a microliter and 25µL of test solution was taken with a micro syringe and placed on a TLC plate. Standard spots of 5 or 10uL of aflatoxin (B1, B2, G1, and G2) were also spotted on the same plate as the internal standard. The standard spots were also made by following the methods of Begum et al. (1985), Romer et al. (1976).

Thin Layer Chromatography Tank

The TLC plate was placed into two TLC tanks of mobile phases. The TLC plate was developed with anhydrous ether in the first TLC tank upto till the mark. After development the plate from tank was removed and then dried. Then the plate was redeveloped with the same direction in the second TLC tank with acetone-chloroform concentration of 1:9. Later

on, TLC plate was removed and left for drying and observed under a UV light Scanner. Fluorescing intensities of sample spots were compared with those of standard aflatoxin spots. In the case of the fluorescing spot of the sample lying between the standard spots, the average value of two standard spots was taken into consideration.

Confirmation of aflatoxins

The levels of aflatoxin were determined with the performance of another very crucial step. The fluorescing of the sample spot was performed during the analysis. At this step, the TLC plate was sprayed with the help of moist Sulphuric Acid. Sulphuric Acid of a 50/50 volume-to-volume ratio was used. After the spray, the TLC plate was allowed to dry and later on, observe under the UV light of a wavelength of 365 nm.

Calculations

The concentrations/levels of aflatoxins detected in sample µg/kg were deliberated by the formula of Saeed et al. (2020).

1. Aflatoxin B1 contents(µg/kg) = S.Y.V / W.Z

S= Standard spot of µl aflatoxin B1 (or any standard)

Y= strength of aflatoxin standard in µg/kg

V= µl of final dilution of sampled extract

W= Weight in g of sample applied ($CHCL_3$) is known as an effective weight

Z= µl sample extract showing fluorescence intensity equal to S

Statistical Analysis

Statistical analysis of samples was carried out by one-way ANOVA and Independent sample T test using SPSS version 21.0.

Results

A total of three feed samples of broiler, quail and ostrich feed were collected from the local shops of Lahore, and Sheikhupura, Punjab, Pakistan. All these samples were quantified using the TLC method for the estimation of aflatoxins.

Detection of aflatoxins in broiler feed samples in the summer and winter season

The broiler feed samples were collected in the summer and winter seasons and processed for the detection of aflatoxins in triplicates. For the summer season sample 2 was found positive for the B1 aflatoxin and sample 3 was found positive both for B1 and B2 samples (Table 1). However, for the winter season, only B1 aflatoxin was detected in sample 3.

Table 1. Detection of aflatoxins in broiler feed samples in the summer and winter season

Broiler feed samples	Aflatoxin	Aflatoxin Detected	Conc. of Aflatoxins(µg/kg) in summer	Conc. of aflatoxins (µg/kg) in winter
Sample 1	B1	-	-	-
	B2	-	-	-
	G1	-	-	-
	G2	-	-	-
Sample 2	B1	B1	25.63±25.66	-
	B2	-	-	-
	G1	-	-	-
	G2	-	-	-
Sample 3	B1	B1	145.50±145.54	15.40±15.36
	B2	B2	46.20±46.23	-
	G1	-	-	-
	G2	-	-	-

Detection of aflatoxins in Quail feed samples in the summer and winter season

The quail feed samples were collected in the summer season and processed for the detection of aflatoxins in triplicates. All three samples were found positive for the B1 aflatoxin, while no sample was found positive for B2, G1, and G2 aflatoxins (Table 2). In the winter season samples, aflatoxin B1 and B2 were detected in samples 2 and 3, respectively.

Table 2. Detection of aflatoxins in quail feed samples in the summer and winter season

Quail feed samples	Aflatoxins	Aflatoxin Detected	Conc. of Aflatoxins (µg/kg) in summer	Conc. of Aflatoxins (µg/kg) in winter
Sample 1	B1	B1	93.70±93.73	-
	B2	-	-	-
	G1	-	-	-
	G2	-	-	-
Sample 2	B1	B1	56.20±56.24	13.49±13.51
	B2	-	-	-
	G1	-	-	-
	G2	-	-	-
Sample 3	B1	B1	64.80±64.84	-
	B2	-	-	11.27±11.31
	G1	-	-	-
	G2	-	-	-

Detection of aflatoxins in Ostrich feed samples in the summer and winter season

The three ostrich feed samples were collected in the summer season and processed for the detection of aflatoxins in triplicates. It was noticed in sample 2 only B1 aflatoxin was detected, while sample three was only positive for G1 aflatoxin (Table 3). In the winter season, aflatoxin B1 was detected only in sample 3.

Table 3. Detection of aflatoxins in Ostrich feed samples in the summer and winter season

Ostrich Feed samples	Aflatoxin	Aflatoxin Detected	Conc. of Aflatoxins (µg/kg) in summer	Conc. of Aflatoxins (µg/kg) in winter
Sample 1	B1	-	-	-
	B2	-	-	-
	G1	-	-	-
	G2	-	-	-
Sample 2	B1	B1	102.90±102.97	-
	B2	-	-	-
	G1	-	-	-
	G2	-	-	-
Sample 3	B1	B1	-	9.07±9.06
	B2	-	-	-
	G1	G1	20.30±20.32	-
	G2	-	-	-

Overall levels of aflatoxins in broiler, quail and Ostrich feed samples in different seasons

Overall levels of aflatoxins were determined from total collected feed samples of the winter and summer seasons. It was noticed mostly quail samples were found contaminated in the summer and winter seasons followed by an equal number of contaminated samples in ostrich and broiler samples. While overall 66.66% of samples were found contaminated irrespective of the summer and winter seasons. The prevalence of each aflatoxin was also

calculated as 81.18% for aflatoxin B1, 18.18% for aflatoxin B2, 9.09% for aflatoxin G1, and 0% for aflatoxin G2.

Prevalence of Contaminated samples beyond the permissible levels

It was noticed all the summer samples were detected with a high percentage of aflatoxins and all were beyond the permissible levels. While all winter samples have aflatoxins within permissible levels.

Discussion

Aflatoxins B1, B2, G1 and G2 are toxic metabolites produced by certain fungi types such as *Aspergillus flavus* and *Aspergillus parasiticus*. The present study was conducted to quantify the aflatoxins (B1, B2, G1, G2) in broiler, quail, and ostrich feed samples. For this purpose, TLC was performed with samples and the same method was used by Wacoo et al, (2014) for the determination of aflatoxins in feeds during the study. Soblev (2007) has also stated that both TLC and HPLC techniques are popular in the detection of aflatoxin levels as the methods have the advantage to determine even in picograms (AOAC).

The present study was carried out with a total of 18 feed samples that were collected in the summer and winter seasons from broiler, quail, and ostrich feed samples. The contamination levels were found as 66.66% while, an overall 79.8% contamination was found with total aflatoxin in feed samples analyzed with ELISA (Dashti et al., 2009). The prevalence of each aflatoxin was also calculated in overall samples irrespective to seasons as 81.18 % for (aflatoxin B1), 18.18 % for (aflatoxin B2), 9.09 % for (aflatoxin G1), and 0 % for (aflatoxin G2) which was very less as compared to another study that reported a prevalence of 82.5% for B1, 69.37 % for B2, 43.12 % for G1, and 41.87 % for G2 were noticed in milk to assess feed (Bahrami et al., 2016).

In the foregoing study, a higher level of contamination was recorded in the summer season as compared to the winter season and a number of studies were found that also report higher concentration in summer as compared to the winter season. One such study has been provided by Nemati et al. (2010) which reported the lowest levels of contamination in winter (17.4 ng/kg) and the highest for the summer seasons (56.3 ng/kg). Another study by Iram et al. (2019) reported the same as our findings that higher levels were found in summer as compared to the winter season in layer feed. Tajkarimi et al. (2008) also reported that seasons affect levels of aflatoxins. Alam et al., (2012) also reported lower levels of contamination were detected in winter as compared to the summer season which was

controversial to our findings. Becha and Devi, (2013) reported in a study that the highest levels of aflatoxins were recorded in the monsoon season as compared to other seasons. In the study, a total of 709 samples were used both from the livestock and poultry sector with the highest prevalence of aflatoxin B1. While, in our study, higher levels of B2 aflatoxin were detected. Anjum et al. (2012) detected aflatoxin B1 (AFB1) in poultry feed samples, the occurrence was 44.39 percent, with average contamination and highest levels of 23.75 and 78 g/kg, respectively. However, the maximum level of AFB1 was higher (78µg/kg) in poultry feed samples. While in another study total of 50 samples of poultry feed were studied with 48 percentage of contamination with the highest level of 16.65 µg/kg Summia et al. (2021). However, in the present study, a higher level of contamination was recorded in all summer samples. In the present study, a higher level of contamination was detected in quail feed followed by ostrich and broiler feed samples while Nakavuma et al. (2022) reported the highest contamination in broiler feed samples. Oliveira et al. (2003) conducted a study where birds were fed with aflatoxin-contaminated feed and later on, its effects were assessed in eggs. The study showed that contaminated feed might produce a higher impact on the body and later becomes part of residual material. Another study also showed intake of contaminated feed in quails affects growth performance and blood profile (Mahrose et al., 2021).

Conclusion

It was concluded in the present study that the percentage contamination of aflatoxins was high in quail, and ostrich feed samples as compared to broiler feed samples. A higher level of prevalence was detected in the summer season as compared to the winter season. The type of aflatoxins B1, B2 and even G1 were also detected in feed samples The highest contamination percentage was detected in sample 3 of the ostrich feed in the summer season and sample 2 of the quail feed as compared to all other feed samples. This study provides knowledge about feed-carrying practices that are not appropriate for different types of birds and emphasized to enhance good storage conditions on farms and with local markets, particularly in summer so that the quality of feed can be improved.

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