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Exploring the Relationship Between Season and Mycotoxins in Poultry Feed and Tissues on Behera Province Farms.

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Abstract

SEASONAL climatic changes significantly affect mycotoxin contamination in poultry feed and tissues, with warmer conditions promoting fungal growth and toxin production. A surveillance study was conducted in Behera Governorate, Egypt, to assess seasonal mycotoxin contamination (specifically, total aflatoxins and ochratoxin A) in broiler feed and tissues, including the liver and kidneys. A total of 300 samples were collected, with 150 samples taken during winter and 150 during summer. This study investigates the seasonal variations in mycotoxin contamination in broiler feed and tissues, evaluates the prevalence of fungi and aflatoxin production, and examines the effectiveness of propionic acid as an antifungal agent to ensure feed safety and protect poultry health. The results indicated higher contamination levels during the summer months. Fungal prevalence was significant across both seasons, with *Aspergillus flavus* identified as the dominant mould, particularly in summer, leading to increased aflatoxin production. The findings underscore the importance of proper storage practices and seasonal monitoring to mitigate fungal growth and reduce mycotoxin risks. Histopathological analyses revealed the toxic effects of mycotoxins, which manifested as severe liver and kidney damage in affected birds. Furthermore, the study demonstrated that a 0.5% solution of propionic acid effectively inhibits mould growth. These results highlight the necessity for antifungal strategies, thorough monitoring, and optimized feed management to ensure feed safety and protect poultry health.

Keywords: Summer, Winter, Aflatoxins, Ochratoxins, Chicken, Pathology, HPLC.

Introduction

Poultry feed is a crucial component of the poultry industry. It consists of mixtures of locally grown cereals, cottonseed meal, pulses, and various additives. Commercial feed mills produce poultry feed. Feed mill owners typically purchase large quantities of ingredients during the production season and store them for year-round feed production. These ingredients are often sourced directly from fields with high moisture content [1].

The warm climate, extended post-harvest period, and humid environmental conditions create an ideal environment for mould growth, which can produce toxins [2]. The poultry industry has become one of the largest sectors in the country, driven by the increasing demand for poultry meat and its by-products. Poultry meat is preferred over meat from larger animals due to its high nutritional value [3].

Despite the widespread commercialization of the industry, poultry birds still face several challenges. Mycotoxicosis is a significant issue, ranking as the second most concerning problem after the rising poultry feed prices [4]. Approximately 300 different mycotoxins have been identified, including aflatoxins, ochratoxins, zearalenone, patulin, and trichothecenes. Among these, ochratoxins and aflatoxins are considered the most critical toxins in the poultry industry [5].

Aflatoxins (AFs) are toxic secondary metabolites produced by Aspergillus species, contaminating agricultural products worldwide [6]. The optimal conditions for aflatoxin production are 33°C and 0.99 water activity, with growth best at 35°C and 0.95 aw [7]. There are approximately 20 types, with B1, B2, G1, and G2 being the most significant [8]. Aflatoxins have been studied for their carcinogenic, mutagenic, teratogenic, immunosuppressive, and growth-

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inhibitory effects in broiler chickens [9]. Contaminated feed can lead to aflatoxicosis in poultry production, which is characterized by various symptoms. These include lethargy, loss of appetite, reduced growth rates, poor feed utilization, decreased egg weight, increased susceptibility to environmental stress and microbial infections, and higher mortality rates [10]. When birds are raised on feed contaminated with toxins, it results in financial losses for farmers and poses health risks to consumers due to the presence of harmful residues. Wild and Gong [11] found significantly higher levels of aflatoxins in the liver compared to the kidney and meat. Furthermore, the International Agency for Research on Cancer (IARC) [12] reported a link between aflatoxin ingestion and liver cancer in humans, particularly in regions of Asia and Africa, as noted in 1993. Therefore, it is crucial to address toxins in animal feed to protect public health.

Several ochratoxins exist, including ochratoxins A, B, and C. Among these, ochratoxin A (OTA) is the most significant and toxic [13]**.** OTA can be found in various products worldwide, such as cereals, animal and poultry feeds, feed ingredients, poultry tissues, wheat, grains, coffee, and chili, as well as animal and human milk and tissues [14]. In poultry, OTA poses several threats to both growing and adult birds. It has nephrotoxic, hepatotoxic, mutagenic, teratogenic, and immunogenic effects. The presence of OTA can lead to decreased feed intake, reduced body weight gain, lower egg production, and changes in the birds' hematological and serum biochemical parameters [15].

Given the toxic and carcinogenic potential of mycotoxins, there is an urgent need to develop rapid and highly specific detection methods. Mycotoxins have diverse chemical structures, making it impractical to create a single method to detect all relevant mycotoxins. Although some progress has been made in developing methods for the simultaneous detection of multiple mycotoxins, it is essential to continue focusing on creating accurate and sensitive techniques for both qualitative and quantitative analysis. [16].

Various studies have examined the factors influencing fungal colonization and mycotoxin production [17]. These factors can be categorized as environmental conditions that promote fungal growth and mycotoxin production, including temperature, relative humidity, and insect infestations. Furthermore, the application of fungicides and fertilizers, alongside stresses such as drought, increased temperatures, and elevated humidity, can selectively modify the colonization and metabolism of mycotoxigenic fungi, affecting mycotoxin production [18].

Antifungal agents can inhibit the growth of mycotoxigenic moulds in cereal commodities. The most effective agents include benzoic acid (0.1%), sorbic acid (0.2%), propionic acid (0.32%), parabens (0.1%), and sodium diacetate (0.32%). Among these, sorbates, propionates, and benzoates, particularly propionic acid, exhibit the greatest antifungal activity and are especially effective in reducing yeast and mould growth [19].

This study analyzed feeds and poultry tissues from farms in Behera Province across two major seasons to demonstrate the impact of environmental climatic conditions on aflatoxin and ochratoxin contamination in chicken feed and tissues. Additionally, the study evaluated the inhibitory effect of propionic acid on the growth of *Aspergillus flavus* and *Aspergillus ochraceus*.

Material and method:

Sampling

A surveillance study was conducted to evaluate the levels of mycotoxin contamination (total aflatoxins and ochratoxin A) in broiler feed and tissues (liver and kidneys) during winter (November to January) and summer (June to August). Three hundred samples (150 per season) were collected from various broiler farms in Behera Governorate, Egypt. The samples were collected using a representative method, as recommended by the FAO, for the detection and determination of aflatoxins and ochratoxin A. The samples were immediately analyzed for mycological examination and then stored in the freezer for toxin analysis and pathological investigation within 2 days.

Isolation of fungi from feed samples

Twenty-five grams of poultry feed samples were aseptically mixed with 225 milliliters of sterile distilled water while well shaken. Afterward, 1.0 milliliter was pipetted into a tube holding 9.0 milliliters of D.W. and gently stirred. The tube for the second dilution was pipetted with 1.0 ml of the first dilution. After this, the liquid was set at room temperature for five minutes, and the process was repeated until a 10-6 dilution [20].

Total mould count

Duplicate dichloran rose bengal agar plates were inoculated with one milliliter of the previously prepared serial dilutions. The inoculation plates were incubated at 25ºC for five days, and the count was recorded on the fifth day [21].

Identification of isolated mould

An isolated mould colony was identified through macroscopic observations of its growth pattern, color, texture, and the characteristics of its basal and surface mycelia. Additional observations included colony reversal, the rate of colony expansion, and the diameter of the colonies. The resulting colonies were then examined microscopically to describe the

morphological structures of the mould growth, using both high- and low-power lenses [22].

Assessment of aflatoxin production with cultural method

The fundamental concept behind aflatoxin biosynthesis in aflatoxigenic A. flavus isolates is the production of yellow anthraquinone pigments, which lead to visible color changes. In the presence of ammonia hydroxide, these yellow pigments turn pink. Isolates were classified as either toxic or non-toxic based on their scores on the testing plates, which could be positive or negative. The strength of the pink color observed varied from intense to no color change at all. A total of 74 A. flavus isolates were cultured on coconut agar medium and incubated in the dark at 30 °C for three days. The intensity of the pink color that developed after exposure to ammonium vapor indicated the level of aflatoxin synthesis. Consequently, a lack of color development signifies that aflatoxin production is absent [23].

Investigation of mycotoxins

The total aflatoxins (sum of B1, G1, B2, and G2) and OTA were estimated using HPLC-FLD [24].

Chemicals

Aflatoxins and ochratoxin A certified references in acetonitrile solution (3µg/ml), and Trifluoroethanoic acid (TFA) were obtained from The Sigma-Aldrich, Steinhaus, Germany. Immunoaffinity cartridges (IAC) for the clean-up step (AFLAPREP® and OCHRAPREP®) are from R-Biopharm Rhône Ltd., UK. Acetonitrile (ACN), Ethanol (EOH), and Methanol (MeOH) of chromatography ultrapure grade, phosphate buffer saline (PBS), and the other used chemicals bought from Merck, Darmstadt, Germany, are analytical grade. and. A Milli-Q-system (Millipore, Mosheim, France) provided deionized water (DW); For the OTA separation, the mobile phase was DW, ACN, and EOH (51:47: 2), and for the AFs analysis, it was DW, ACN, and MeOH (60:20:20).

Sample preparation

The extraction process followed the method outlined by Youssef et al. [24] Three grams of minced tissue or feed were mixed with 0.3 grams of sodium chloride and 10 mL of a solution containing 45% acetonitrile and 55% distilled water (ACN: DW) for 10 minutes. The mixture was then centrifuged for 2 minutes at 3000 rpm at room temperature. Two mL of the filtered liquid was mixed with 2 mL of distilled water for the extraction of AFs, while the extraction of OTA was done using 2 mL of PBS. The sample was slowly passed through a specific immunoaffinity cartridge at a flow rate of 1 drop per second, followed by a wash with 1 mL of water at the same flow rate. Elution was carried out with 1 mL of MeOH for AFs and OTA residues. The elute was then evaporated at 40°C under a nitrogen stream. For OTA analysis, the residues were dissolved in 600 µL of the mobile phase before injection into HPLC. For AF analysis, derivatization was required. After drying, derivatization of AFs was carried out by adding 100 µl of TFA in a dark environment for 15 minutes at room temperature with the vial caps on. Finally, 500 µL of a mixture of ACN: DW (1:9) was added to the vials.

Apparatus and separation conditions

The HPLC system consists of an autosampler injector, a quaternary pump (model 1200), and a fluorescence detector (FLD) manufactured by Jasco Corporation in Tokyo, Japan. A volume of hundred microliters was injected into the LC system (Agilent 1200, USA) at a flow rate of 1 ml/min. For separation, a HypersilTM BDS C18 column from Thermo Fisher Scientific is utilized. The column measures 250 mm in length, with an inner diameter of 4.6 mm and a particle size of 5 µm. It operates at 40°C and is equipped with FLD from Japan. The excitation and emission wavelengths used were 365 and 442 nm for aflatoxins and 333 and 463 nm for OTA.

Intra-lab verification of the analytical method

Verification criteria, including linearity, recovery, precision, the limit of detections, and quantifications (LOD and LOQ) were established following ICH [25] and USP [26] requirements. Method precision was determined through six replicated analyses of fortified samples, with inter-day precision measured over 6 days and repeatability (intra-day precision) at the quality control level of 1.5 ppb. The calibration plot for all examined toxins ranged from 0.15-6 ppb. Recovery was assessed by fortifying blank feed and tissues at three levels: 1/2X, 1X, and 2X the QC level.

Histopathological examination

To determine the histopathological changes, samples from the liver and kidney were collected carefully and rapidly placed in 10% neutral buffered formalin then after 48 h. fixed tissues were processed in ascending grades of alcohol, cleared by xylene, and finally embedded in paraffin wax to prepare 5-µm-thick sections stained with hematoxylin and eosin (HandE) stain for microscopic examination [27].

Determination of the antifungal effect of propionic acid

Poultry ration samples were analyzed for mycological examination and have higher mould count stored in the refrigerator for addition of propionic acid as antifungal. Three samples have the same higher count, first sample was used as a control positive (+ve), second sample was sterilized in autoclave for 30 min. then inoculated on Dichloran rose Bengal agar plates to confirm that it free from

fungi, then 2nd sample was divided into 3 parts and inoculated by propionic acid 0.3%, 0.4%, and 0.5%. 3rd sample was divided into 3 parts and inoculated by propionic acid 0.3%, 0.4% and 0.5% as described by Haque et al. [28]. All samples incubated in room temperature for one month and examined weekly to evaluate the effectiveness of propionic acid on total mould.

Statistical analysis

Data were expressed as mean ± S.D. using the commercially available software package (SPSS Inc., version 22.0, Chicago, IL, USA) for data analysis [29].

Results

Prevalence of fungi from feed samples

The isolated fungi from feed samples were tabulated in Table 1, showing that the prevalence of fungal contamination in feed samples was 92% and 88% for the summer and winter seasons, respectively.

Total mould counts in the analyzed ration samples

The results presented in Table 2 indicate that the total mould count of the ration samples examined during the winter season was higher than that of the samples examined in the summer season. Specifically, the mean mould count was 12 x 10^2 cfu/g for the winter season and 10 x 10^2 cfu/g for the summer season.

The most isolated mould species from the examined ration sample was

The results presented in Table 3 indicate that samples collected during both the summer and winter seasons were contaminated with various fungal genera. In the summer, the most predominant mould genera isolated were *Aspergillus flavus* (38%), *A. niger* (18.9%), and *Mucor* spp. (17%). In contrast, the predominant isolates found in the winter samples were *Aspergillus flavus* (29.1%), *A. niger* (16.2%), and *Cladosporium cladosporioides* (11.1%).

Assessment of A. flavus strains for aflatoxin production

Table 4 shows that 25% of the isolated *A. flavus* strains collected in the summer were high aflatoxin producers, while only 14.7% of the strains collected in the winter were high producers. In contrast, the non-producing aflatoxin strains accounted for 42.5% of the total examined strains in the summer and 64.7% in the winter (Figure 1).

The results of the quality control assessment for the chromatographic method

Table 5 presents the results of the mycotoxins method qualification. Figure 2 illustrates a linear relationship in the range of 0.15-3.0 ppb for all analyzed mycotoxins, with a correlation coefficient of over 0.99. The retention times (RT) of the Afla-G2, -G1, -B2, -B1, and OTA standards are 0.451, 0.591, 1.071, 1.979, and 11.856 minutes, respectively (Figures 3, 4).

Results of the detected mycotoxins in the analyzed samples

Table 6 demonstrates that during summer months, the total aflatoxin levels in the collected feed samples varied from 5 to 417 ppb, with a 54% occurrence. In contrast, the range was 2.8 to 35 ppb during winter with a 38% occurrence. For liver samples, the total aflatoxin levels ranged from 1.5 to 16.84 ppb, with a 28% occurrence in summer months, and from 0.08 to 1.4 ppb, with a 16% occurrence in winter (Table 7). Table 8 indicates that the ochratoxin-A in the collected feed samples ranged from 94 to 500 ppb, while the kidney samples ranged from 1.21 to 6.3 ppb.

Histopathological examination

P.M. examination

At the post-mortem dissection, the livers of birds suspected of mycotoxin exposure were grossly enlarged, pale in color, with some dark spotted areas, and friable during handling. The kidneys were also pale and enlarged.

Microscopic examination

Livers showed hepatocellular degeneration, cytoplasmic vacuolation, cloudy swelling, and central and portal vein congestion. There is mononuclear cell infiltration in hepatic tissue as a part of the inflammatory response Figure (5). While kidneys showed hydropicrenal tublar epithelial cells degeneration, glomerular and renal blood vessel congestion, and infiltration of inflammatory cells on renal interstitial tissue Figure (6).

Determination of the antifungal effect of propionic acid

Table 9 demonstrates the effect of propionic acid on the growth of fungi. Also, the inhibition rate of propionic acid was more superior with increasing concentrations. Increasing the propionic acid concentration to 0.5% resulted in a greater inhibitory effect on the total mould count. Furthermore, the lowest concentration of propionic acid was delayed and decreased mould growth. The count in 1st week 1.5×10^4, 7.5×10^3, 3.0×10^3 and 2.5×10^2, Then count in 4th week 4.3×10^8, 7.5×10^2, 3.0×10^2 and 1.1×10^2 in control (+ve) and contaminated ration sample with 0.3%,0.4% and 0.5% propionic acid, respectively. In the control ration sample (-ve) with propionic acid delayed mould growth.

Discussion

The analysis of fungal contamination in feed samples collected during both summer and winter seasons highlights significant aspects of fungal

ecology and the factors influencing fungal growth and prevalence. The overall prevalence of fungal contamination in feed samples was notably high, at 92% during the summer and 88% during the winter. This indicates a persistent presence of fungi in animal feed throughout the year, albeit with a slight reduction during the colder months. The high prevalence in summer can be attributed to favorable environmental conditions such as higher temperatures and humidity levels, which promote fungal growth as Temeche et al. [30] who discussed the persistent presence of fungi in animal feed throughout the year, with higher prevalence in summer due to favorable environmental conditions such as higher temperatures and humidity levels.

Interestingly, despite the higher prevalence of fungal contamination in summer, the total mould count was higher in winter. Specifically, the mean mould count was 12 x 10^2 cfu/g for winter and 10 x 10^2 cfu/g for summer. This could be due to several factors. Winter conditions, characterized by high relative humidity and cooler temperatures, can create a conducive environment for the growth of certain mould species, leading to a higher mould count. Additionally, feed storage practices during winter might be less effective in controlling moisture and ventilation, resulting in increased mould growth. Different fungal species have varying optimal growth conditions, and those predominant in winter might have higher growth rates under cooler, more humid conditions Ng'ang'a and Niyonshuti [31] mentioned that different environmental conditions, such as high relative humidity and cooler temperatures in winter, can create a conducive environment for the growth of certain mould species, leading to a higher mould count. It also mentions that feed storage practices during winter might be less effective in controlling moisture and ventilation, resulting in increased mould growth.

The fungal genera isolated from the feed samples varied between the summer and winter seasons. In the summer, the predominant genera were *Aspergillus flavus* (38%), *Aspergillus niger* (18.9%), and *Mucor* spp. (17%). *Aspergillus flavus* is particularly concerning due to its ability to produce aflatoxins, which are highly toxic and carcinogenic. *Aspergillus niger* produces ochratoxins, which are nephrotoxic, while *Mucor spp*. are less commonly associated with mycotoxins but can still pose health risks. During the winter, the predominant genera were *Aspergillus flavus* (29.1%), *Aspergillus niger* (16.2%), and *Cladosporium cladosporioides* (11.1%). *Cladosporium cladosporioides* is notable for its ability to grow at lower temperatures and its potential to cause allergic reactions and respiratory issues as mentioned by Ng'ang'aand Niyonshuti [31] who provided valuable insights into the ability of *Cladosporium cladosporioides* to grow at lower temperatures and its potential to cause allergic reactions and respiratory issues.

The presence of mycotoxin-producing fungi like *Aspergillus flavus* and *Aspergillus niger* in both seasons poses significant health risks to animals and humans. Mycotoxins can lead to reduced feed quality, impaired animal health and productivity, and potential transfer into the food chain. The data underscores the need for tailored feed management strategies for different seasons. During summer, strategies should focus on controlling moisture levels and temperature, while winter strategies should ensure proper ventilation and storage to minimize fungal growth [30, 31]. Regular monitoring of feed for fungal contamination and mycotoxin levels is essential. Implementing effective antifungal treatments and using mycotoxin binders can help mitigate the risks associated with fungal contamination.

The results of the method qualification for mycotoxins are essential for ensuring the reliability and accuracy of the analytical method employed. The method shows a linear relationship within the concentration range of 0.15 to 3.0 ppb for all analyzed mycotoxins, with a correlation coefficient exceeding 0.99. This high correlation coefficient indicates a strong linear relationship between the concentration of mycotoxins and the detector response, which is crucial for precise quantification [32]. The retention times (RT) for the standards of Aflatoxin G2, G1, B2, B1, and Ochratoxin A (OTA) are 0.451, 0.591, 1.071, 1.979, and 11.856 minutes, respectively. These retention times are critical for the identification and quantification of mycotoxins in samples, as they help in distinguishing between different mycotoxins based on their elution times during chromatographic separation [33].

Early separation in High-Performance Liquid Chromatography (HPLC) assays is essential for ensuring the analysis's purity, accuracy, and efficiency [34]. By achieving early separation, we can minimize the time needed for analysis, reduce solvent consumption, and enhance the resolution of analytes, leading to more reliable results. Additionally, early separation aligns with the principles of green chemistry, which aim to lessen the environmental impact of chemical processes. Optimizing separation conditions helps to minimize the use of hazardous chemicals and waste, thus supporting more sustainable analytical practices. Implementing eco-friendly strategies in HPLC, such as using environmentally friendly solvents and reducing energy consumption, not only benefits the environment but also improves the overall efficiency and cost-effectiveness of the analysis [35]. Early separation in HPLC assays is crucial for achieving accurate and efficient results while promoting environmentally responsible analytical practices following green chemistry principles.

The data indicates a significant seasonal variation in aflatoxin levels, with higher levels observed during the summer months. This could be due to increased temperatures and humidity, which are conducive to the growth of *Aspergillus* fungi that produce aflatoxins. The warmer conditions during summer provide an ideal environment for fungal growth and aflatoxin production, resulting in higher contamination levels in feed samples [36].

The occurrence rates of aflatoxins in feed samples are higher in summer (54%) compared to winter (38%), suggesting that aflatoxin contamination is more prevalent during warmer months. This prevalence is likely driven by the favourable conditions for fungal proliferation and toxin production during the summer [37, 38, 39].

Nji et al. [38] noted that Africa experiences significant mycotoxin contamination in foods, contributing to high liver cancer rates. The agricultural sector heavily relies on climate, making mycotoxin levels sensitive to climate variability. Many stakeholders in the food production chain remain unaware of the health and economic impacts of contaminated foods. This review identifies six key factors linked to high mycotoxin levels in African foods, with climate change being the primary driver. Other primarily human-made factors can be managed to improve profitability and reduce climate sensitivity. Raising awareness about mycotoxins and their effects is crucial, as management strategies differ across regions.

Aflatoxins are known to be hepatotoxic and carcinogenic, posing significant health risks to animals. The higher levels of aflatoxins in feed during summer could lead to increased exposure and potential health issues in livestock, such as liver damage, reduced growth rates, and immunosuppression. Continuous exposure to high levels of aflatoxins can also lead to chronic health problems and decreased animal productivity [40].

Ochratoxin-A levels in feed samples are also higher compared to kidney samples, indicating that this mycotoxin is more prevalent in feed. Ochratoxin-A is nephrotoxic and can cause kidney damage in animals, leading to impaired kidney function and other related health issues. The presence of ochratoxin-A in feed highlights the need for stringent monitoring and control measures to ensure animal health and safety [41].

Although mould counts tend to be higher in winter, aflatoxin levels are lower during this season. This suggests that factors such as storage conditions and feed composition may significantly influence aflatoxin production. The increased mould counts in winter might be due to inadequate storage conditions; however, the colder temperatures and lower humidity levels do not favor aflatoxin production. This implies that not all moulds found in the feed produce aflatoxins, and the specific conditions required for toxin production are generally not met during winter [42].

Histopathological changes in the liver, including congestion of the central and portal veins, as well as vascular and hydropic degeneration of hepatocytes, are associated with the infiltration of inflammatory cells in the renal interstitial tissue of the hepatic parenchyma, consistent with findings by Tahir et al. [43]. Additionally, Bakeer et al. [44] reported that dietary supplementation of ochratoxin caused congestion of the cortical blood vessels and intertubular capillaries in the kidneys of broiler chickens. The renal glomeruli were occasionally enlarged due to the proliferation of endothelial cells in the glomerular capillaries.

Hashem and Mohamed [45] explain that degenerative changes in the liver result from damage to lipids, DNA, and proteins due to the oxidative stress caused by aflatoxins. This leads to lipid peroxidation and oxidative DNA damage. Aflatoxicosis triggers mitochondrial dysfunction and reduces ATP generation. The kidneys exhibited congested blood vessels, glomerular corpuscles, hydropic degeneration of renal tubule epithelium, and aggregation of inflammatory cells in renal interstitial tissue, which is consistent with the findings of Del Bianchi et al. [46], Karaman et al. [47], and Tessari et al. [48].

To inhibit mould growth in feed samples and, consequently, the production of mycotoxins, the addition of propionic acid is recommended. Sodium propionate and calcium propionate are authorized for use in food within the EU. The European Food Safety Authority (EFSA) [49] has confirmed that propionic acid occurs naturally as a by-product of normal intermediate metabolism, which means that residues in meat, milk, or eggs are expected to be negligible. Therefore, the use of propionic acid and its salts in animal nutrition is considered safe for consumers.

Increasing the propionic acid concentration to 0.5% resulted in a greater inhibitory effect on the total mycelium growth of the mould species. Our results are consistent with those of Ramadan et al. [50], who reported that formic acid (H-CO-OH), acetic acid (CH3-CO-OH), and propionic acid (CH3-CH2-COOH) showed the highest inhibitory effects on the growth of fungi. As the concentration of propionic acid increased, there was a corresponding decrease in the mould growth rate. The weak organic acid propionic acid is an important preserver in food and feed and inhibits growth of various **y**east and fungi [51].

Conclusions

This surveillance study highlights the seasonal variations in mycotoxin contamination in broiler feed

and tissues, with higher contamination levels observed during summer. Fungal prevalence was significant across seasons, with *Aspergillus flavus* as the dominant mould, particularly in summer, contributing to higher aflatoxin production. The findings also revealed that proper storage practices and seasonal monitoring are crucial in mitigating fungal growth and mycotoxin risks. Histopathological analyses confirmed the toxic effects of mycotoxins, showing severe liver and kidney damage in affected birds. Furthermore, the study demonstrated the efficacy of 0.5% propionic acid in significantly inhibiting mould growth. It has to be expected that propionic acid exhibits both fungistatic and fungicidic effects on the moulds. These findings underscore the importance of integrating antifungal strategies, rigorous monitoring, and optimal feed management to enhance feed safety and safeguard poultry health.

Recommendations

The study recommends addressing seasonal variations in mycotoxin contamination, with a focus on managing higher levels during summer. Emphasis should be placed on proper storage practices and seasonal monitoring to control fungal growth and mycotoxin risks. Effective antifungal strategies, such as incorporating 0.5% propionic acid to inhibit mould growth, particularly *A. flavus*, should be adopted. Additionally, optimizing feed management practices is essential to ensure feed safety and protect poultry health from the toxic effects of mycotoxins.

Acknowledgment

Not applicable.

Conflict of interest

According to the authors, there isn't a conflict of interest.

Funding statement

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Ethical approval

According to ethical guidelines.

TABLE 1. Prevalence of mould genera in examined ration samples in summer and winter.

Type of sample	Total No. of examined samples.	+ve Samples		-ve Samples	
		No	%	No.	%
Poultry ration (Summer)	50	46	92	4	8
Poultry ration (Winter)	50	44	88	6	12

TABLE 2. Total mould counts cfu/g in ration samples.

Total mould count Season	Minimum	Maximum	Mean ±SE.
Summer	3X10	$12x10^3$	$(10x10^2) \pm (3x10^2)$
Winter	1x10	$15x10^3$	$(12x10^{2)} \pm (4x10^2)$

TABLE 3. Prevalence of different mould species isolated from ration samples in both summer and winter seasons.

Season	Summer		Winter	
Mould Spp.	No.	%*	No.	%*
A. candidus	1	0.9	3	2.7
A. carbonarius	1	0.9	0	00
A. clavatus	2	1.9	2	1.7
Acremonium strictum	5	4.7	2	1.7
A. flavus	40	38.0	34	29.1
A. fumigatus	0	00	2	1.7
A. gluacus	0	00	3	2.7
A. niger	20	18.9	19	16.2
A. penicilloides	0	00	1	0.8
A. restrictus	0	00	2	1.7
A. terreus	2	1.9	2	1.7
A. ustus	1	0.9	1	0.8

Cladosporium Cladosporioides.	3	2.8	13	11.1
Cladosporium Sphaerospermum	5	4.7	2	1.7
Curvularia pallescens	0	00	1	0.8
Fusarium spp.	0	00	3	2.7
Mucor spp.	18	17.0	12	10.3
P. aurantiogriseum	1	0.9	1	0.8
P. brevicompactum	1	0.9	1	0.8
P. citrinum	1	0.9	5	4.3
P. corylophilum	0	0	1	0.8
P. grisofulvum	1	0.9	1	0.8
P. simplicissimum	2	1.9	2	1.7
P. subturcoseum	2	1.9	4	3.4
Total	106	100	117	100

*Percentages were calculated based on the total number of isolated mould species from the samples examined.

TABLE 4. Aflatoxin-producing*A. flavus* strains detected with cultural methods.

A. flavus	**+++ve**		**++ve**		**+ve**		**-ve**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Summer strains (40 strains)	10	25	8	20	5	12.5	17	42.5
winter strains (34 strains)	5	14.7	3	8.8	4	11.8	22	64.7

TABLE 5. Sheet of intra-lab quality control for analyzed mycotoxins

	AFB1	**AFB2**	**AFG1**	**AFG2**	**OTA**
RT (min.)	1.979	1.071	0.591	0.451	11.856
Range (ppb)			0.15- 3.0		
Correlation coefficient (R^2)	0.99976	0.99973	0.99958	0.99986	1.0000
LOD (ppb)	0.01174	0.020657	0.016275	0.015522	0.001473
LOQ (ppb)	0.03522	0.061972	0.048824	0.046567	0.004418

TABLE 6. No. of positive samples for aflatoxins and ochratoxin A in feed and tissues during different seasons

	AFT				**OTA**			
	Feed		**Liver**		**Feed**		**Kidney**	
	S	W	S	W	S	W	S	W
Number	27	19	14	8	19	9	10	6
Incidence%	54	38	28	16	38	18	20	12

TABLE 7. Total Aflatoxins concentrations (ppb) in feed and liver samples (n=50)

		Afla B1		Afla B2		Afla G1		Afla G2		Total AF		
		S	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	***p-value***
	Mean	33.59	8.3	6.02	0.92	17.97	3.1	11.6	0.9	48	9.8 *	
	±SD	74.2	.7.2	5.9	0.48	39.59	2.9	20.3	0.85	100	9.4	
Feed	Minimum	1.3	2.6	1.1	0.1	0.2	0.6	0.4	0.1	5	2.8	0.04
	Maximum	346	18	18.7	1.3	135	8	71	1.8	417	35	
	Occurrence %	54	38	14	10	22	14	24	6	54	38	
	mean	3.4	0.5	1.5	nd	2.4	0.14	0.4	nd	4.68	0.6*	
	sd	2.4	0.5	0.4	nd	2.8	0.05	0.3	nd	4.3	0.5	
Liver	minimum	1.2	0.08	1.23	nd	0.5	0.1	0.14	nd	1.5	0.08	0.02
	maximum	7.1	1.4	1.8	nd	7.8	0.17	0.6	nd	16.84	1.4	
	Occurrence %	28	16	4	nd	12	4	4	nd	28	16	

TABLE 8. Ochratoxin-A concentrations (ppb) in feed and kidney samples

		OTA		
		S	**W**	***p-value***
	Mean	16.8	1.29*	
	sd	21.9	1.87	
Feed	Minimum	2.3	0.01	0.035
	Maximum	96	7.2	
	Occurrence %	38	12	
	Mean	1.18	0.3*	
	sd	0.6	0.2	
Kidney	Minimum	0.2	0.1	0.023
	Maximum	2.1	0.7	
	Occurrence %	20	12	

TABLE 9. Antifungal effect of propionic acid

Time	***Control ration sample (+ve)**	****Control ration sample (-ve)**			**Contaminated ration sample**		
		0.3% P. A	**0.4% P.A.**	**0.5% P.A.**	**0.3% P.A**	**0.4% P.A.**	**0.5% P.A.**
1st week	1.5×10^4	0	0	0	7.5×10^3	3.0×10^3	2.5×10^2
2nd week.	2.3×10^5	0	0	0	5.1×10^3	2.6×10^3	2.3×10^2
3rd week.	5.1×10^6	>10	0	0	9×10^2	3.0×10^2	2.1×10^2
4th week	4.3×10^8	>10	0	0	7.5×10^2	3.0×10^2	1.1×10^2

*Control ration sample(+ve) = ration free from propionic acid

**Control ration sample (-ve) = ration sterilized in autoclave

Fig. 1. Coconut agar media shows +ve Aflatoxin production (A) and -ve Aflatoxin production (B)

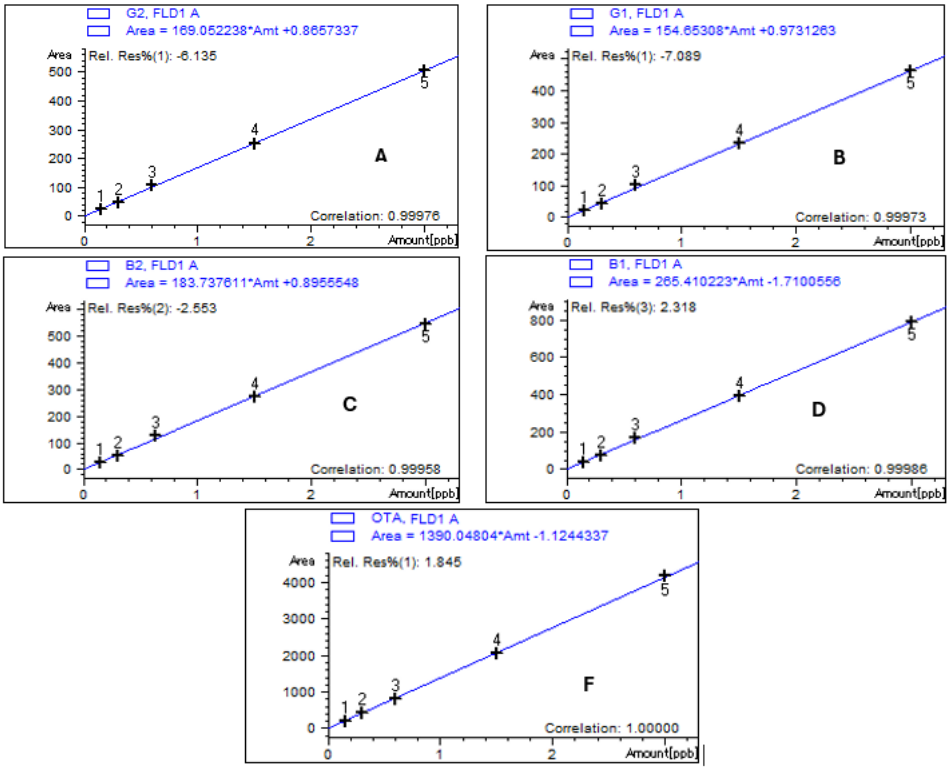

Fig. 2. Standard curves for Aflatoxins (A-D) and OTA (F).

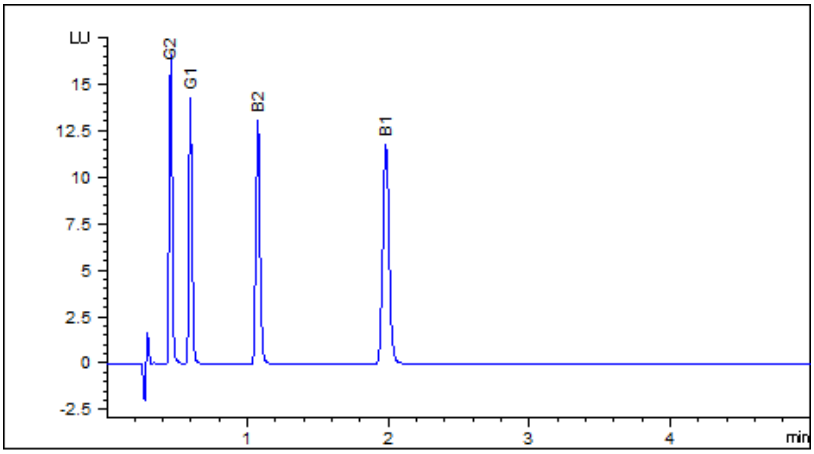

Fig. 3. Chromatogram of aflatoxins standard at a concentration of 0.15 ppb

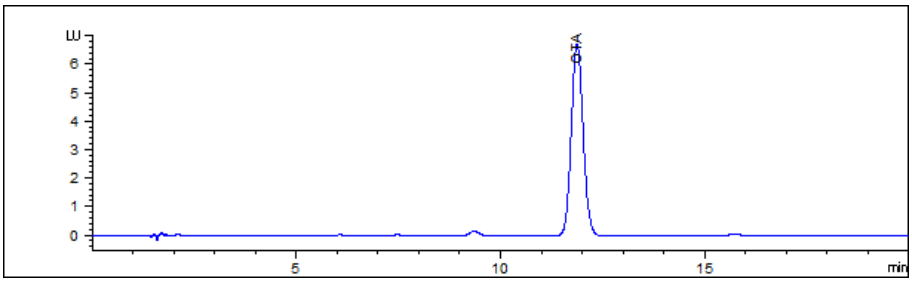

Fig. 4. Chromatogram of ochratoxin-A standard at a concentration of 0.15 ppb

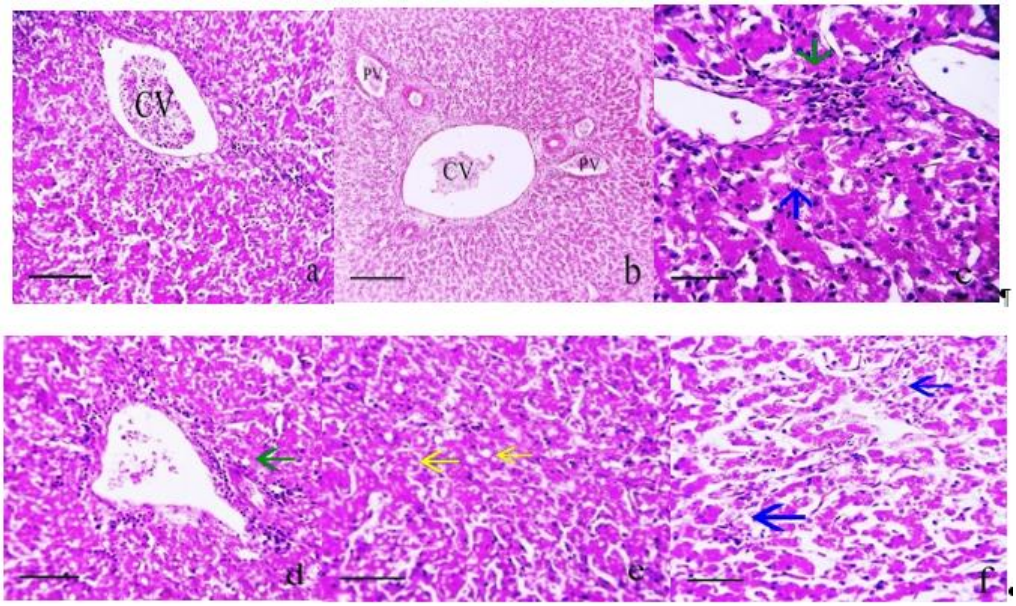

Fig. 5. Liver of chicken showing congestion of central vein(CV), portal vein(PV) (a,b) (HandE,X100) with infiltration of inflammatory cells (blue arrows) (c,d) (HandE,X250,100 respectively). vacuolar degeneration and cloudy swelling of hepatocytes (green and yellow arrows) (e,f)(HandE,X100).

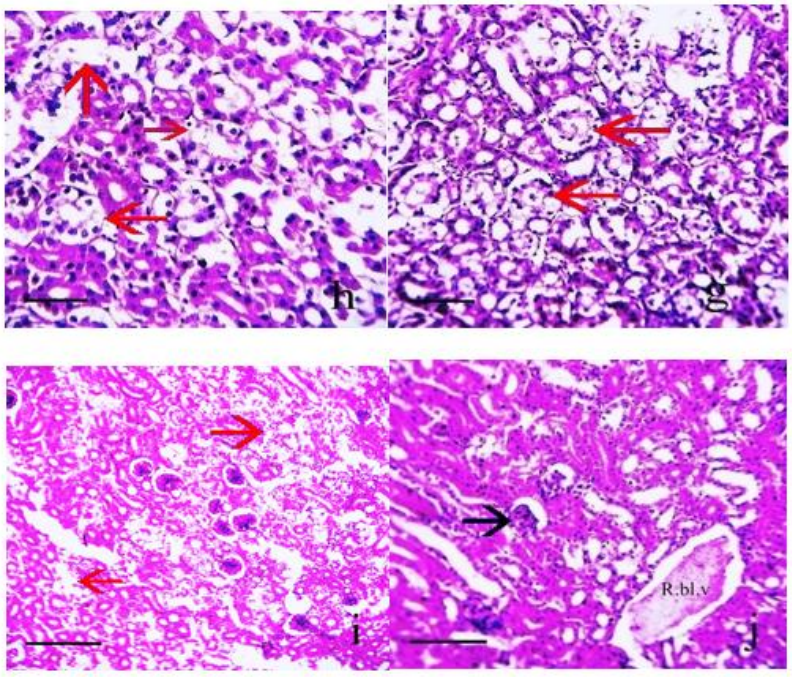

Fig. 6. Kidneys of chicken showing hydropic degeneration and necrosis of the renal tubular epithelium and some glomeruli (red arrows and black arrow respectively)(g,h) (HandE, X250). Congestion of renal blood vessels (R.bl.v) and congested renal glomerular tuft (green arrows) (I, j) (HandE, X100).

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البحث في العلاقة بين العوامل الموسمية ومستويات السموم الفطرية في علف الدواجن بمزارع محافظة البحيرة

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الملخص

تؤثر التغيرات المناخية الموسمية بشكل كبير على تلوث أعلاف وأنسجة الدواجن، حيث تعزز الظروف الدافئة نمو الفطريات وإنتاج السموم. أجريت دراسة مراقبة في محافظة البحيرة بمصر لتقييم تلوث السموم الفطرية الموسمي (وتحديدًا، إجمالي الأفلاتوكسينات والأوكراتوكسين أ) في أعلاف وأنسجة دجاج التسمين، بما في ذلك الكبد والكلى. جُمعت 300 عينة، منها 150 عينة خلال الشتاء و150 عينة خلال الصيف. تبحث هذه الدراسة في التغيرات الموسمية في تلوث السموم الفطرية في أعلاف وأنسجة دجاج التسمين، وتُقيّم انتشار الفطريات وإنتاج الأفلاتوكسين، وتفحص فعالية حمض البروبيونيك كعامل مضاد للفطريات لضمان سلامة الأعلاف وحماية صحة الدواجن. أشارت النتائج إلى ارتفاع مستويات التلوث خلال أشهر الصيف. كان انتشار الفطريات كبيرًا في كلا الموسمين، حيث تم تحديد فطر *Aspergillus flavus* باعتباره العفن السائد، وخاصة في الصيف، مما أدى إلى زيادة إنتاج الأفلاتوكسين. تؤكد النتائج أهمية ممارسات التخزين السليمة والمراقبة الموسمية للحد من نمو الفطريات وتقليل مخاطر السموم الفطرية. وكشفت التحاليل النسيجية المرضية عن الآثار السامة للسموم الفطرية، والتي تجلى تأثيرها في تلف شديد في الكبد والكلى لدى الطيور المصابة. علاوة على ذلك، أظهرت الدراسة أن محلول حمض البروبيونيك بتركيز 0.5% يثبط نمو العفن بفعالية، تُبرز هذه النتائج ضرورة اتباع استراتيجيات مضادة للفطريات، والمراقبة الدقيقة، وإدارة مُحسّنة للأعلاف لضمان سلامتها وحماية صحة الدواجن.

الكلمات الدالة: راكتوبامين، ناهض بيتا، HPLC، اللحوم، الطهى.