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Research Article

Detection of Mycotoxins in Poultry Eggs

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Abstract

Background and objective: Safe food is imperative for food production worldwide. Poultry eggs are crucial in the safe food chain; thus, special attention is directed towards the possible contamination of food and feed with fungi and the risk of mycotoxin contamination. This study aimed to identify the presence of Fumonisin (FU) and Zearalenone (ZON) in 100 laying poultry eggs in Diyala province. **Materials and Methods:** This study was carried out using high-performance liquid chromatography (HPLC). A total of 100 egg samples were randomly collected from different poultry farms in Diyala province. Data were analyzed using ANOVA. **Results:** The results obtained from the analysis of mycotoxins FU and ZON in the poultry egg samples were 94 and 82%, respectively. **Conclusion:** These findings indicate that there may be a risk for animal exposure to mycotoxins through the consumption of mold-infected feeds, which may affect consumer health.

Key words: Fumonisin, fungal contamination, fusarium, mycotoxins, zearalenone

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The secondary metabolites produced by fungi during the course of digestion are called mycotoxins. Fungi produce mycotoxins for a wide array of reasons, many of which remain unknown¹. Mycotoxin production tends to increase when fungal growth rates slow down and fungi move towards dormancy; in such instances, mycotoxin production appears to be a defensive reaction². Alternatively, fungi may produce mycotoxins to protect dormant molds and fungal spores from other surviving fungal species and bacteria. Perhaps mycotoxins help protect molds from adverse environmental conditions, such as extremely cold or dry conditions, or from the lack of essential nutrients in the substrate on which the mold is growing³.

Laying hens are susceptible to mycotoxins for a couple of reasons. The longer rearing period (70 weeks or more) makes them prone to chronic mycotoxicosis. This can be further exacerbated by the increased use of byproducts in layer diets, which can contain up to three times more mycotoxins than grains. Greco *et al.*⁴ found that contamination by mycotoxins occurs frequently in chicken feeds and although more than 500 mycotoxins have been characterized, the most significant ones from the commercial layers' perspective are Aflatoxin, Ochratoxin, Vomitoxin (DON), T-2 toxin, Zearalenone and Fumonisin⁵.

The ingestion of mycotoxin-contaminated feed by chickens causes several health problems and leads to large economic losses in terms of egg quality and quantity. The common mycotoxins found in eggs are Aflatoxin, Ochratoxin, Zearalenone and Fumonisin⁶. In addition, a combination of Aflatoxin and Zearalenone in feeds was shown to synergistically reduce the laying performance, egg quality and feed intake of laying hens⁷.

Mycotoxins affect the bottom line of layer operations through many routes, including the effects on egg production, egg weight, eggshell thickness, leg weakness and immunity. The optimum egg shell quality in layers is critical to control nutrient losses, reduce bacterial contamination and increase the shelf life of eggs^{8,9}. Shell integrity is the parameter of egg shell quality most commonly affected by mycotoxins, which can also affect the shape, texture and cleanliness of eggs¹⁰.

However, despite the importance of eggs as a nutritious and beneficial commodity, there is little information on co-occurring mycotoxin contaminations in poultry eggs in Iraq. Therefore, this research was conducted to detect mycotoxin in poultry eggs in Diyala province.

MATERIALS AND METHODS

Research was conducted in coordination with the laboratories of the College of Veterinary Medicine/University of Baghdad and the Iraqi Ministry of Science and Technology.

Collection of samples: A total of 100 egg samples were randomly collected from various laying poultry farms in Diyala province from November 2016 to March 2017. Samples were kept on ice during their transportation to the laboratory where they were kept at 4°C until analysis.

Chemicals: All reagents and solvents were HPLC or analytical grade. Standards of FU and ZON were purchased from Sigma. The analysis was performed on an Agilent 1200 Series HPLC with a diode array detector (DAD). The analytical column was an Agilent ZORBAX Eclipse Plus C18 2.1 mm × 100 mm, 1.8 μm. An Agilent 0.22 μm nylon syringe filter was used to filter the sample solution before HPLC.

Extraction and cleanup of sample: Ten grams of egg samples without eggshell was homogenized with a blender for 5 min at room temperature and placed into a 100 mL centrifuge tube with 40 mL 84:16 (v/v) acetonitrile:water. Samples were then homogenized for 3 min with a high speed homogenizer and centrifuged at 10,000 r/min for ten mins at 4°C. The supernatant was completely evaporated under a stream of nitrogen at 50°C. The residue was redissolved in 8 mL 50:50 (v/v) ethyl acetate/cyclohexane and passed through a 0.45 μm nylon filter. Then, 20 μL was subjected to HPLC analysis¹¹.

Statistical analysis: All data were analyzed using a one-way ANOVA test. Differences were considered significant at $p \leq 0.05$. SPSS (version 22) was used for statistical assessments.

RESULTS

The results obtained from the analysis of mycotoxins (FU and ZON) in the laying poultry egg samples are presented in Table 1. The predominant mycotoxin for all analyzed samples was FU. The incidence of FU and ZON in all the samples was 94 and 82%, respectively. No significant differences were found between the median FU and ZON contents for samples ($p < 0.05$).

Table 1: HPLC data for the occurrence of mycotoxins in poultry egg samples

Mycotoxins	Positive samples	Incidence (%)	Range ($\mu\text{g kg}^{-1}$)	Mean \pm SD* ($\mu\text{g kg}^{-1}$)
FU	47	94	1.52-2.98	2.13 \pm 0.90 ^a
ZON	44	88	0.87-2.76	1.64 \pm 0.85 ^a

DISCUSSION

The progress of poultry production is a result of diverse technological developments in nutrition and management techniques. However, significant economic losses may occur due to the presence of natural feed contaminants such as mycotoxins, which are secondary metabolites produced by fungi that naturally grow in cereals and other grains. Mycotoxins influence the metabolism of poultry by reducing the activity of the enzymes that digest starch, proteins, lipids and nucleic acids, decreasing blood protein, total cholesterol and urea and increasing the activity of serum enzymes that indicate liver damage¹². The main manifestations of mycotoxin infection in layers are reduced egg production, reduced weight and increased liver fat levels¹³.

Of the main egg components (yolk, albumen and shell), the yolk has the longest development time. Precursors to yolk lipoproteins are produced in the liver and transported through circulation to the yolk follicles in the ovary. In an active laying hen, several follicles at varying developmental stages reside simultaneously in the ovary. Before an egg is laid, the yolk undergoes a stage of rapid growth, during which it increases in size exponentially over 10 days¹⁴. Mycotoxins that deposit in the yolk will rapidly accumulate during this time and can be presented in successive eggs for 10 or more days following treatment. Following yolk maturation, the albumen, or "egg white," is laid down over a period of 2-3 h and can also serve as a residue accumulation site. Residues are found in egg albumen at low levels and in egg yolks at similar concentrations to those found in kidney and liver for several days following oral dosing¹⁴.

CONCLUSION

These findings indicate that there may be a risk for animal exposure to mycotoxins through the consumption of mold-infected feeds, which may affect consumer health.

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