

# MONITORING THE PRESENCE AND INVESTIGATION OF TOXIGENIC FUNGI AND MYCOTOXINS IN POULTRY FEED AND ITS PRODUCTS

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## Abstract

Contaminating poultry feed and their products with mycotoxins produced by fungi may cause many health effects on animals and human if they were at high concentrations. Therefore, it is imperative to regularly monitor the concentration of mycotoxins specially aflatoxin and ochratoxin A in the poultry feed and their products. In the present study, we demonstrated that *Aspergillus flavus* was the major contaminant. Ochratoxin A did not exceed the detection limit 50 ng/kg but in one sample has exceeded the European Union maximum limit for aflatoxins of 20 µg/kg. Aflatoxin b1 was detected in chicken liver samples. Almost all samples were contaminated with fungi but only 4 feed samples showed aflatoxin concentration within the detection limit.

## Literature Review

Mycotoxins are mainly produced by *Aspergillus*, *Fusarium*, *Rhizopus*, *Alternaria* and *Penicillium* (Joshaghani *et al.*, 2013). Some species of fungi produce these mycotoxins which are toxic metabolites that are secreted and found in feed, food and food products. Approaching 300 to 400 mycotoxins are indicated (Pereira *et al.*, 2019). There most leading mycotoxins are aflatoxins (AFs), zearalenone (ZEA), ochratoxin A (OTA), trichothecenes (TRCs) and fumonisins (FMs) (Rai *et al.*, 2015). Some of the mycotoxins are identified as strong carcinogenic agents like AFs B1. The intake of food having mycotoxins may lead to mycotoxicoses which are diseases caused by such intake (Rai *et al.*, 2015).

## Objectives

- 1- Explore the presence of mycotoxins in poultry feed samples in Qatar.
- 2- Isolate and identify toxigenic fungi from poultry feed samples.
- 3- Explore the genes encoding mycotoxins and production potential of the isolated fungi.

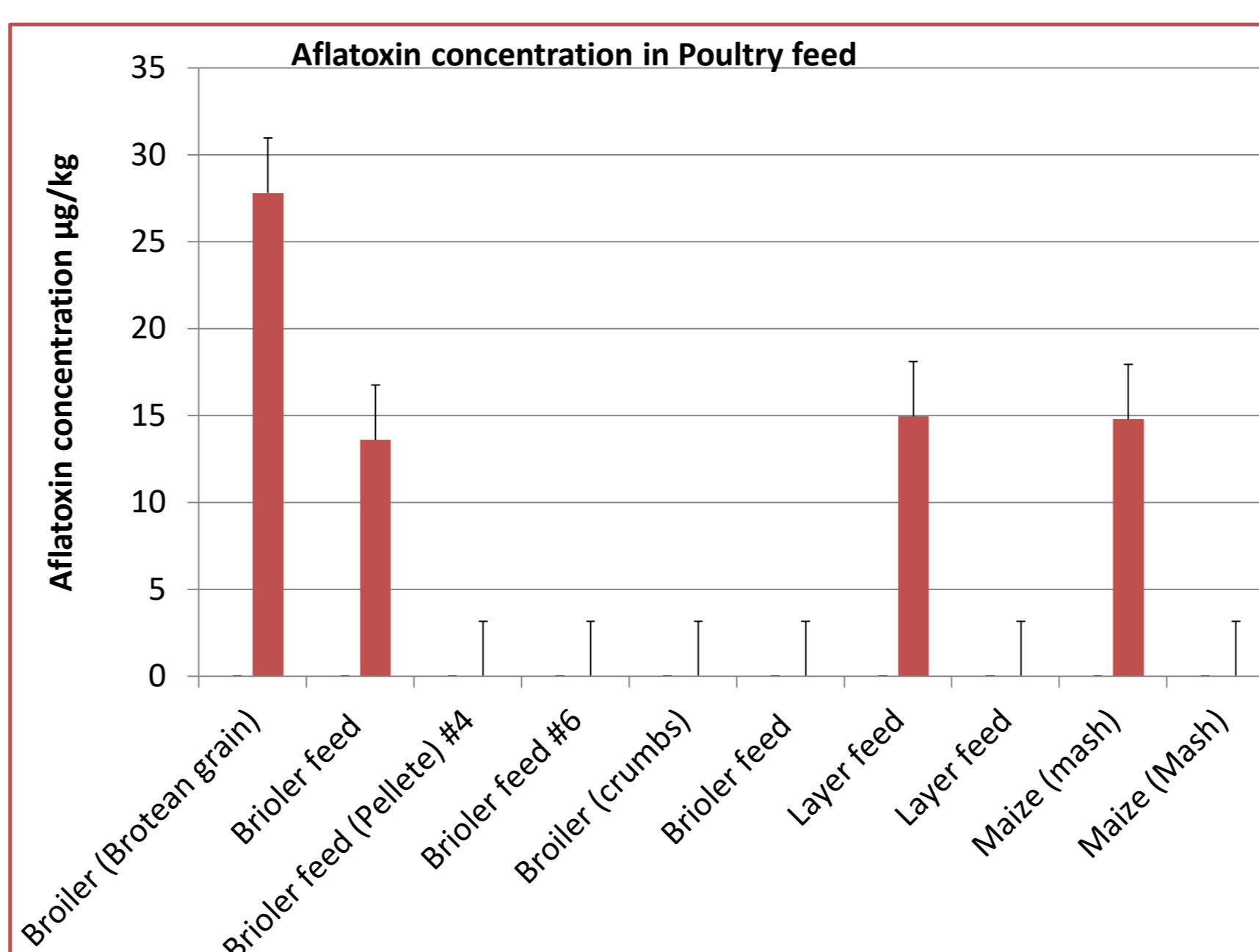
## Materials and Methods

**Materials:** Growth media DRBC, PDB, PDA, YES. Primers TS1, TS4, PAR1, PAR2, FLA1, FLA2, CAR1, CAR2, NIG and Gene-specific primers (OMT-208 – OMT-1232, APA-450 – APA-1482 and VER-496 – VER-1391, AoOTA-L – AoOTA-R) PCR, ELISA plate reader, plant DNA extraction kit.

**Methods:** Fungal species were isolated and identified by inoculating on DRBC, MEA and PDA. DNA was extracted using the plant DNA extraction kit and PCR is used for the molecular identification of isolates primers PAR1/PAR2, FLA1/FLA2, CAR1/CAR2, ITS1/NIG. Then, detect aflatoxigenic genes (APA, VER and OMT) Aflatoxin level was detected using total aflatoxin ELISA kit and Ochratoxin A was detected by Ochratoxin A ELISA kit. Finally, ELISA assay was used for mycotoxins quantification of pure isolates.

## Results and Discussion

### 1. Poultry feed samples are contaminated with Aflatoxins



Aflatoxin contamination in Poultry feed samples

- Aflatoxin was detected in 2 Maize, 1 layer feed and 1 broiler feed samples out of 10 samples
- Maize (Brontean grain) presented highest aflatoxins level (27.8 µg/kg) followed by layer and broiler feed.

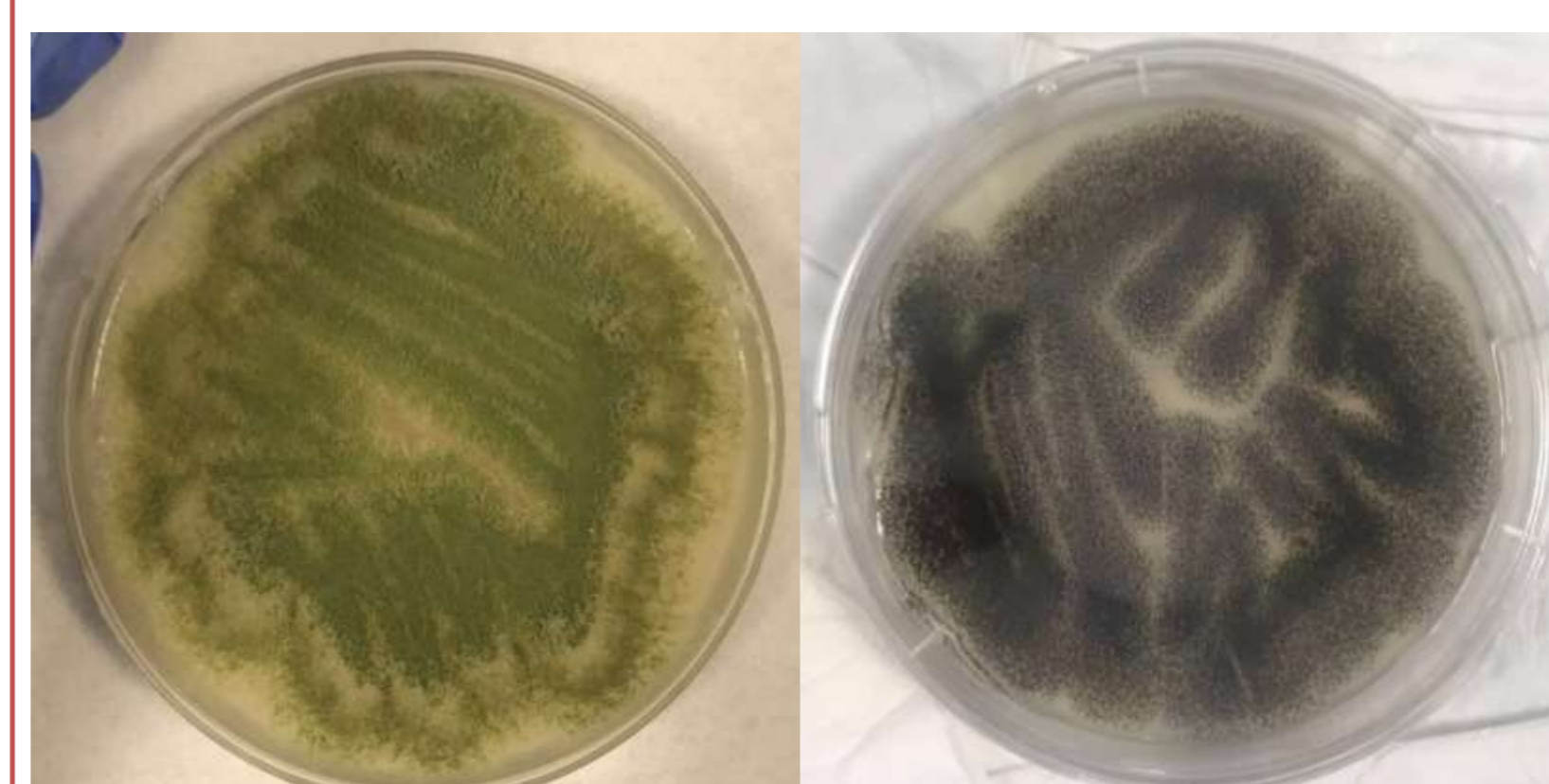
### 2. Several fungi were isolated from Poultry feed samples



Fungal contamination of poultry feed samples

- Selective and differential screening of fungal isolates from Poultry feed samples using selective and differential fungal media.
- Different colonies where isolated to be identified.

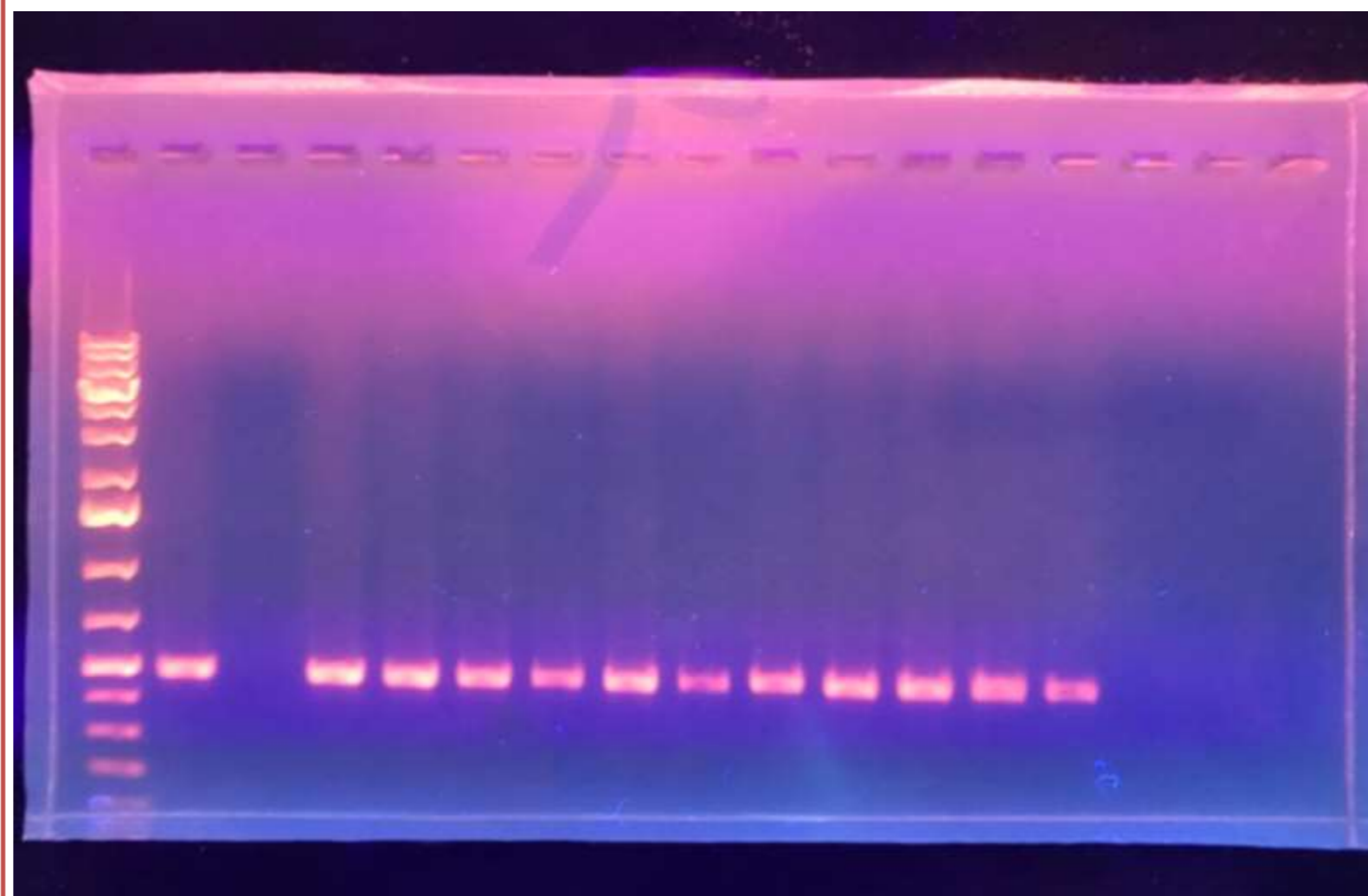
### 3. Morphological identification of aflatoxigenic and Ochratoxigenic fungi, isolated from poultry feed samples



A. Flavus and A. Niger on PDA

- Morphological identification through (size, shape, color, sporulation, form, elevation) of aflatoxigenic and Ochratoxigenic fungi.
- Among total 17 isolates, 3 were A. Niger and 1 A. creaceous, 12 were identified either A. flavus or A. parasiticus, since the two species are very morphologically similar.

### 4. Molecular identification of fungal isolates

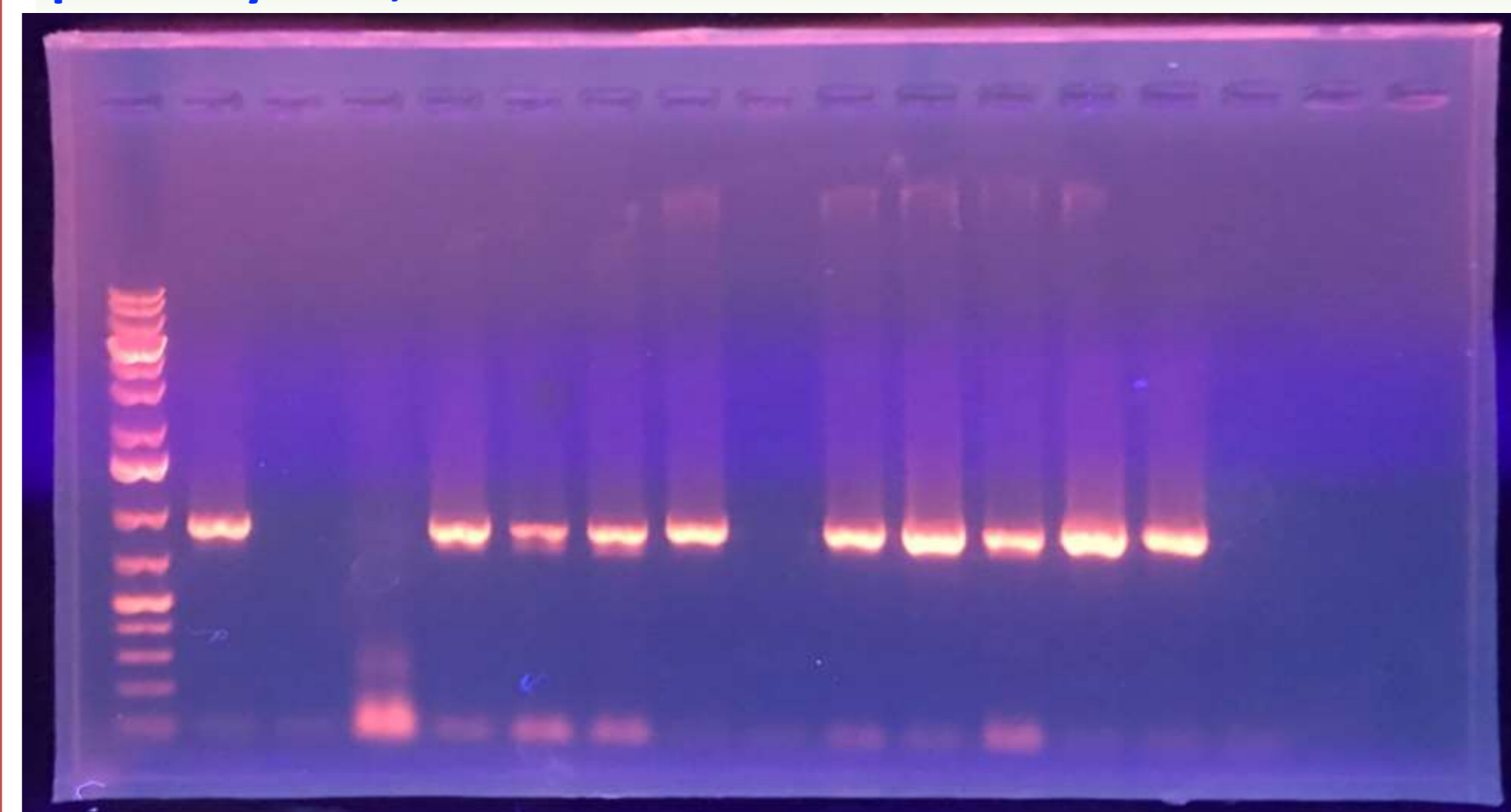


PCR amplification of DNA from fungal isolates, using the primers Fla1/Fla2 (amplicon size 500 bp).

- Primer Fla1/Fla2 were used to confirm that the analysed clones are of A. Flavus.

### 5. Identification of Aflatoxigenic genes

OMT, APA and VER primers were used to confirm the presence of the genes of the aflatoxin biosynthesis pathway: aflP, aflR and aflM.

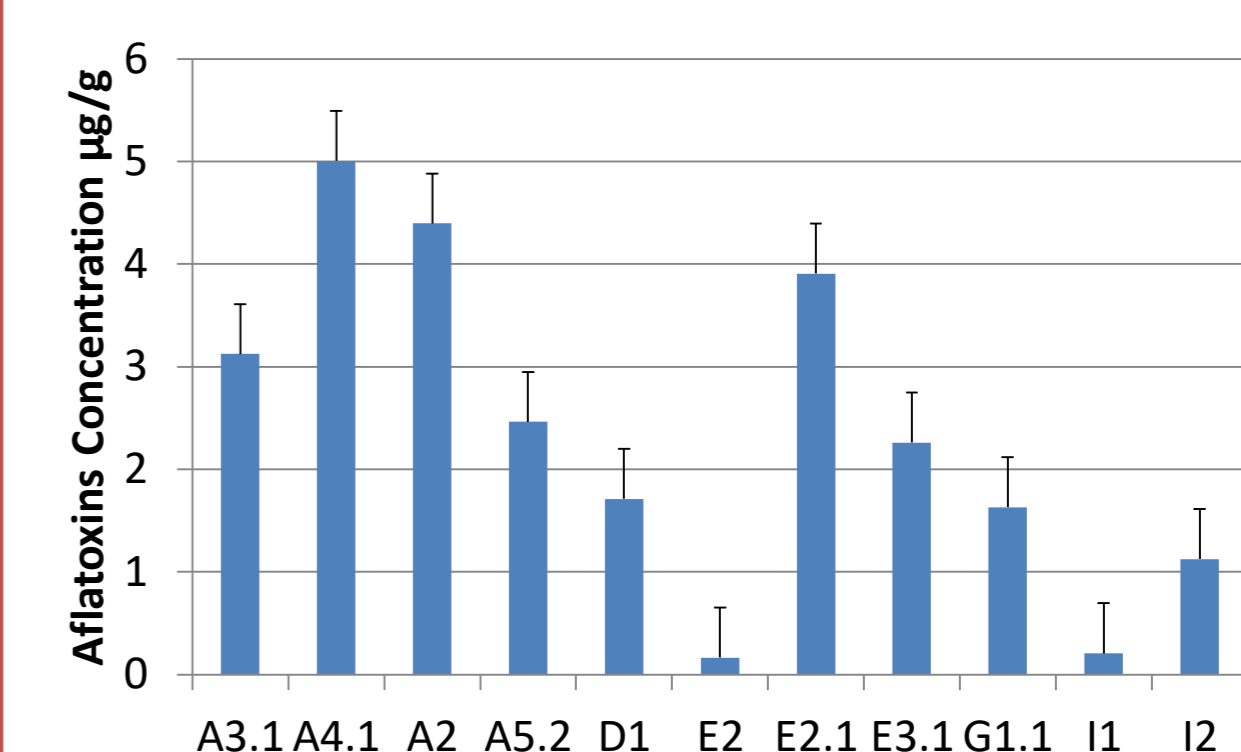


PCR amplification of DNA from fungal isolates, using the primers VER1/VER2 (amplicon size 895 bp).

1: 100 bp ladder, 2-PCR products of positive control, 3 negative control, others: fungal samples

### 6. Aflatoxin production potential of fungal strains isolated from poultry feed

Aflatoxin Production for Isolates

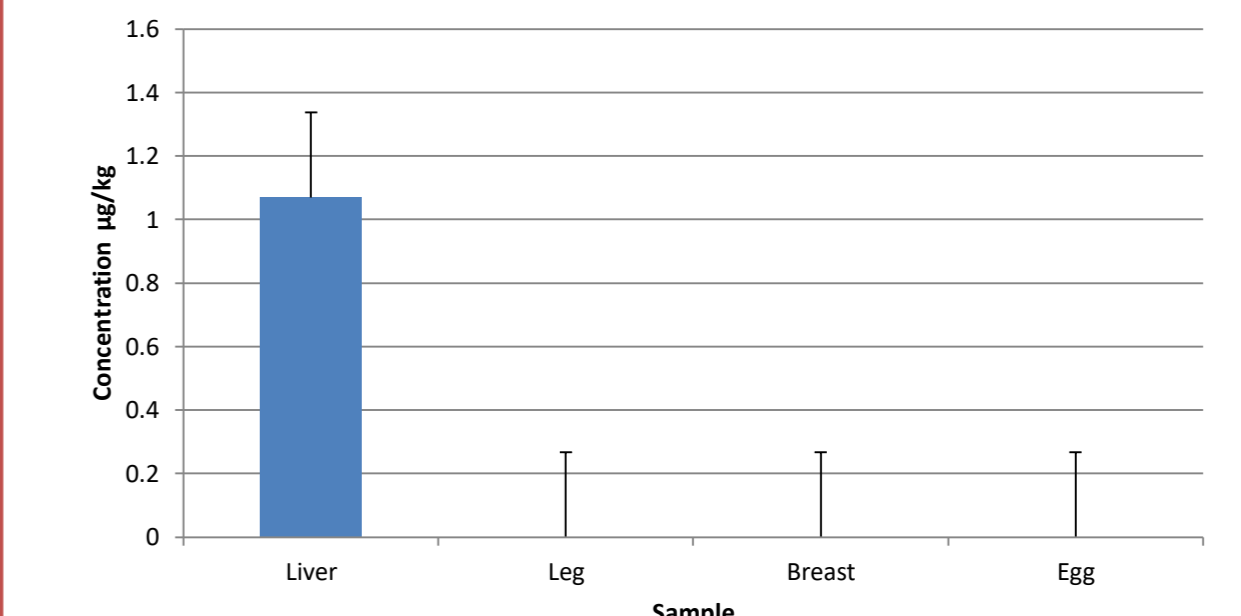


- Aflatoxins production varied between the different samples

Aflatoxins Concentrations in isolated fungi from poultry feed.

### 7. Detection of Aflatoxin B1 in Meat and eggs

Aflatoxin B1 Concentration in Poultry meat and egg



- Liver samples showed Alatoxin B1 concentration average of 1.07 µg/kg
- Leg, breast and egg showed negative results for aflatoxin B1

Concentrations of aflatoxin B1 in Poultry liver, leg, breast and egg.

## Conclusion

On the basis of our findings, it can be concluded that:

- 1- The explored Poultry feed samples were contaminated with mycotoxins.
- 2- *Aspergillus flavus* is the most abundant fungus in the Poultry feed samples.
- 3- The synthesis of mycotoxins was confirmed by the presence of the corresponding coding genes in the fungal isolates.
- 4- Chicken liver is contaminated with aflatoxin B1.

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