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# MYCOFLORA AND NATURAL OCCURRENCE OF MYCOTOXINS IN LIVERS OF IMPORTED BULLS AND POULTRY AND SOME MEAT PRODUCTS

(With 2 Tables)

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## الميكوفلورا والسموم الفطرية المتواجدة في أكباد كل من العجول المستوردة والدواجن وبعض منتجات اللحوم

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يعد التلوث الغذائي من أهم المشكلات في الوقت الحالي الأمر الذي دفعنا لعمل دراسة عن مدى تواجد الميكوفلورا وبقايا الميكوتوكسين المختلفة في بعض منتجات اللحوم مثل اللنشون واللحوم المفرومة وكذلك أكباد العجول المستوردة والدواجن. وقد تم تجميع ٤٠ عينة بواقع عشر عينات لكل نوع وبطريقة عشوائية من المطاعم والسوبرماركت المختلفة بمحافظة أسيوط. وقد تم عزل ٢٩ نوع من الفطريات المختلفة وقد تراوح العدد الكلى للفطريات اكل جرام ما بين ٢٦٨٠ بالنسبة للنشون إلى ٧٤٦٠ بالنسبة لأكباد الدواجن. وكان فطرى الاسبرجلس والبنسليوم على التوالي من اكثر الفطريات انتشارا حيث تم عزلهم من جميع العينات. وقد أوضحت نتائج مدى تواجد متبقيات الميكوتوكسين عن تواجد أربع أنواع مختلفة وهى الافلاتوكسين $B_1$ , $B_2$ , $G_1$ و$G_2$ والاوكراتوكسين A والسترنين والسترجماتوسيستين. كم أظهرت الدراسة أن هذه المتبقيات متواجدة في ٤٥% من مجموع العينات وبنسبة أعلى من المعدل المسموح به وأن أعلى معدل كان في أكباد العجول المستوردة ( ٥٤ ، ١٤٥ جزء في البليون بالنسبة للافلاتوكسين والاوكراتوكسين A على التوالي). الأمر الذي يشكل خطورة بالغة على صحة المستهلك خاصة على فترات طويلة .

## SUMMARY

Mycological analysis of local meat products (luncheon and minced meat) and livers of poultry and imported bulls resulted in isolation of 29 fungal species related to 10 genera. The average total counts of fungi per gram fresh weight ranged from 2680 in luncheon to 7460 in livers of poultry.

*Aspergillus* was the most prevalent genus followed by *Penicillium* where they were isolated from all the examined substrates. Many of the isolated fungi might have mycotoxin-producing potential. Results of mycotoxins analysis revealed that, 45% of the examined samples were positive. Aflatoxins ($B_1$,$B_2$,$G_1$ and $G_2$), Ochratoxin A, Citrinin and Sterigmatocystin were detected. Samples of livers from imported bulls contained the highest levels of aflatoxins and ochratoxin A (54 and 145 µg/kg, respectively). The majority of the remaining mycotoxin contarninated samples contained a high level which was far above the acceptable ones. The hazardous effects of these natural pollutants were discussed.

***Key words:*** *Mycoflora, mycotoxins, livers, poultry, meat products.*

## INTRODUCTION

Contamination of meat products with moulds leads to great economic losses, besides it constitutes a major public health hazard. The contamination of meat products by species of *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor* and *Cladosporium* were reported by many investigators (Torrey and Marth, 1977; Hefnawy, 1980; Zohri, 1990; Zaki et al., 1995 and Nagat, 1997).

Mycotoxins, as secondary metabolites produced by many strains of moulds in different food and food products are highly toxic, non immunogenic, potent carcinogens and causes a potential hazard to human health, (Youssef et al., 1986 and Bahgat, 1999). Humans are exposed to mycotoxins directly by consuming contaminated commodities or indirectly by consuming animal products or organs have ingested mycotoxins contaminated feeds (Hsieh, 1981).

Therefore the present study was designed to study the fungal contamination and assay for the natural occurrence of several myctoxins in most popular meat products (Luncheon and minced meat) and edible organs (Livers of poultry and imported bulls).

## MATERIALS and METHODS

**Samples:**

Ten random samples of each of luncheon, minced meat, livers of poultry and livers of imported bulls were collected from different localities in Assiut City. Each sample (200 gm) was put in a sterilized

polyethylene bag. Samples were transferred immediately to the laboratory and each sample was divided into 2 parts. One part was kept in a deep freezer (-20°C) untill mycotoxin analysis while the other part was used for mycological analysis.

**Mycological analysis:**

This was made by using the dilution-plate method as described by Christensen (1963). A desired final dilution which supports a total of about 20-40 colonies per plate was used.

A modified glucose-Czapek's agar medium which contained: $NaNO_3$, 3; $KH_2PO_4$, 1; $MgSO_4$, 0.5; KCl, 0.5; $FeSO_4$, 0.01; glucose, 10 and agar, 15 (gm/L of distilled water) was employed in this study. Rose bengal was used as a bacteriostatic agent (Smith and Dawson, 1944).

One ml of the desired dilution was transferred aseptically into sterile Petri-dish and 20-25 ml of the medium; cooled to just the solidification; were added. Three replicates of each sample were prepared. The dishes were incubated at 28°C for 7 to 10 days during which the growing colonies were identified and counted. The identification of fungi was according to De Vries (1952); Domsch and Gams (1972); Raper and Fennel (1965), and Raper and Thom (1949)

**Mycotoxin analysis:**

25 gm of each sample were homogonized with 100 ml of chloroform for 5 min. in a high speed blender. Extraction was repeated three times. The combined chloroform extract was washed by distilled water, dried over anhydrous sodium sulphate, filtcrated and concentrated to near dryness on a rotary evaporator. The residue was diluted with chloroform to one ml. The chloroform solution was analyzed for the presence of aflatoxins, ochratoxins, citrinin, sterigmatocystin, zearalenone, $T_2$-Toxin and patulin using thin-layer chromatographic procedures (Gimeno, 1979)

The aflatoxin content was analysed and confirmed using trifluoroacetic acid derivative formation (A.O.A.C., 1984). Citrinin and ochratoxin A were quantively determined according to Scott et al. (1972) and Nesheim et al. (1973), respectively while sterigmatocystin quantity was determined by the method described by Schroeder and Kalton (1975).

## RESULTS

The obtained results are recorded in Tables 1 and 2

## DISCUSSION

Mycological analysis of local meat products (Luncheon and minced meat) and livers of poultry and imported bulls resulted in isolation of 29 fungal species related to 10 genera. The average total counts of fungi (per gm fresh weight) were 2680,7340, 7460 and 4760 in luncheon, minced meat, livers of poultry and imported bulls respectively. *Aspergillus* was the most prevalent genus followed by *Penicillium* where they were isolated from all the examined substrates. The remaining isolated genera were *Alternaria, Cladosporium, Fusarium, Mucor* and others (Table, 1). Most of these genera were previously isolated but in different frequencies from chicken, meat and meat products in Egypt ( Hefnawy, 1980; Hegazi et al., 1992; Zaki et al., 1995 and Nagat, 1997).

From *Aspergillus*, 13 species were identified of which *A. flavus* was the most common. This fungal species was emerged in high occurrence from minced meat and livers of imported bulls, while it was moderatly occurred in luncheon and livers of poultry. *A. terreus* and A. niger were isolated in high frequencies from minced meat and livers of poultry, while *A.ochraceous* and *A.parasiticus* were isolated from 30% of luncheon and livers of imported bulls respectively (Table, 1).*Penicillium* incidence ranged from moderate in minced meat to low in luncheon, livers of poultry and livers of imported bulls. 4 *Penicillium* species were identified of which *P.viridicatum* was isolated from 30% of livers of imported bulls and 20% of both luncheon and minced meat (Table, 1). Nearly similar results were reported by Yassien et al., (1990), Salem (1991), and Zaki et al. (1995). In a similar study, *Aspergillus* and *Penicillium* were recorded as the predominant genera in minced meat, luncheon and pastirma (Abdel-Rahman et al., 1984).

Regarding the results of mycological analysis of the examined samples (Table 1), many of the isolated fungi may have mycotoxin producing potential. Therefore, the presence of mycotoxins in these samples could be excepted. Mycotoxins can enter the food supply by direct contamination resulting from mould growth on the food, or indirectly through the use of contaminated ingredients in processed foods or by feeding mouldy feeds to food producing animals. In previous study, poultry feed ingredients were found to contain high levels of aflatoxins and Zearalenone (Mahmoud, 1993). Indirect contamination of food may be a problem in some area of the world where food is more

highly processed (Bullerman, 1979; Bullerman et al., 1984; Zohri, 1990 and Bahagt, 1999).

In the present study, 4 types of mycotoxins were detected of which aflatoxins were predominant and liver samples had the highest percentage of contamination (Table 2). Aflatoxins ($B_1$, $B_2$, $G_1$ and $G_2$) were detected in 4 samples of livers from imported bulls with a mean concentration of 54µg/kg. In addition to aflatoxins, ochratoxin A was found in one sample with concentration of 145µg/kg. Mycological analysis of these samples showed that 90% and 30% of them were contaminated with *A. flavus* and *A. parasiticus*, respectively. Similar findings were reported by Bullerman (1979) and Refai (1988). Bullerman et al. (1984) reported that the liver is the critical animal tissue most likely to contain aflatoxin residues. On the other hand, Zaki et al. (1993) did not detect aflatoxins in liver, kidney and muscles of baldy bulls. This variation in results may be due to the fact that the level of aflatoxins contamination in feedstuffs is important factor influencing the tissue aflatoxin residues.

Citrinin was detected in 4 samples of both minced meat and livers of poultry with mean concentration of 103 and 108 µg/kg, respectively. It is worth to mention that, in the citrinin contaminated samples, aflatoxins were also detected in 3 and 2 samples of minced meat and livers of poultry with mean concentration of 23 and 36µg/kg, respectively. While sterigmatocystin was detected in one minced meat sample, with concentration of 115 µg/kg (Table 2). These results are in agreement with those of Hegazi et al. (1992) who found that 33% of minced meat samples contained aflatoxins. In a similar study, Bullerman (1979) observed that aflatoxins were mainly accumulated in the liver of poultry, duckling and turkey and could cause liver damage. No literatures are available about the contamination of minced meat and livers of poultry with citrinin. We believe that this is the first report in this respect. The high level of citrinin in minced meat and livers of poultry may be attributed to the high occurrence of *A.terreus* on these substrates (Table 1).

40% of luncheon samples were contaminated with aflatoxins and ochratoxin A with mean concentration of 20 and 100 ug/kg, respectively. Mixed contamination was detected in one sample. Such results agreed with those recorded by Zohri (1990) and Zaki et al., (1995). Mycotoxins contamination of luncheon may be originated either from the animal tissues previously fed on mycotoxins contaminated feed, or due to using

mycotoxins contaminated ingredients e.g. cereals and spices (Zaki et al., 1995).

The results of the present study have shown that the most examined samples were relatively highly contaminated by fungi and mycotoxins. The majority of mycotoxins contaminated samples contained a high levels which far above the acceptable ones (Bullerman, 1979 and Bahagt, 1999). Thus efforts have to be made to prevent mould growth and mycotoxins production along the entire food chain, from field to table. Another very important points in meat plants before mass slaughtering (especially in cases of imported bulls or hen's flock) representative numbers of apparently healthy animals must be analysed for occurrence of mycotoxins residues which if found by levels more than 20 ug/kg, slaughter should be delayed and the feeding of animals on mycotoxins free diets for 3-4 weeks as sufficient with holding period to clear the muscles and organs from toxins ( Kerogh et al., 1976 a).

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Table (1): Average total counts, number of cases of isolation (out of 10 samples) and occurrence remarks of fungal genera and species isolated from luncheon, minced meat and livers of poultry and imported bulls.

| Fungal genera and species | Luncheon | | | Minced meat | | | Livers of poultry | | | livers of cattle | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ATC | N.C.I | OR | ATC | N.C.I | OR | ATC | N.C.I | OR | ATC | N.C.I | OR |
| *Alternaria alternata* | 100 | 2 | L | 140 | 2 | L | 180 | 2 | L | - | - | - |
| *Aspergillus* | 1820 | 6 | H | 5880 | 10 | H | 6300 | 7 | H | 4400 | 10 | H |
| *A. candidus* | - | - | - | - | - | - | 60 | 3 | L | - | - | - |
| *A.flavipes* | - | - | - | 40 | 1 | R | 160 | 2 | L | - | - | - |
| *A. flavus* | 740 | 4 | M | 1740 | 8 | H | 420 | 4 | M | 3180 | 9 | H |
| *A.fumigatus* | - | - | - | 380 | 4 | M | 440 | 4 | M | 380 | 3 | L |
| *A.nidulans* | 140 | 1 | R | - | - | - | 20 | 1 | R | - | - | - |
| *A. niger* | 220 | 3 | L | N.980 | 6 | H | 940 | 6 | H | 240 | 2 | L |
| *A.ochraceus* | 360 | 3 | L | oc.260 | 1 | R | 140 | 1 | R | - | - | - |
| *A. oryzae* | - | - | - | 260 | 1 | R | - | - | - | - | - | - |
| *A.parasiticus* | - | - | - | - | - | - | - | - | - | 200 | 3 | L |
| *A.sydowi* | - | - | - | - | - | - | 60 | 1 | R | 400 | 5 | M |
| *A.tamarii* | - | - | - | - | - | - | 260 | 3 | L | - | - | - |
| *A.terreus* | 220 | 2 | L | 1980 | 8 | H | 3480 | 7 | H | - | - | - |
| *A.versicolor* | 140 | 1 | R | 300 | 3 | L | 300 | 3 | L | - | - | |

Table 1 continued

| Fungal genera and species | Luncheon | | | Minced meat | | | Livers of poultry | | | livers of cattle | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ATC | N.C.I | OR | ATC | N.C.I | OR | ATC | N.C.I | OR | ATC | N.C.I | OR |
| ***Cladosporium*** | **80** | **1** | **R** | **440** | **5** | M | 400 | 3 | L | - | - | - |
| *C.cladosporioides* | **80** | **1** | **R** | **340** | **4** | M | 400 | 3 | L | - | - | - |
| *C.herbarum* | - | - | - | **100** | **1** | R | - | - | - | - | - | - |
| ***Cohiolubolus spicifer*** | **40** | **1** | **R** | - | - | - | - | - | - | - | - | - |
| ***Drechslera spicifera*** | - | - | - | **40** | **1** | R | - | - | - | - | - | - |
| ***Fusarium*** | **80** | **1** | **R** | **380** | **4** | M | 260 | 4 | M | 40 | 1 | R |
| *F. Oxysporum* | - | - | - | **180** | **2** | L | - | - | - | - | - | - |
| *F.verticillioides* | **80** | **1** | **R** | **200** | **2** | L | 260 | 4 | M | 40 | 1 | R |
| ***Mucor*** | **40** | **2** | **L** | **180** | **2** | L | - | - | - | - | - | - |
| *M. circinilloides* | - | - | - | **80** | **1** | R | - | - | - | - | - | - |
| *M.hiemalis* | **40** | **2** | **L** | **100** | **1** | R | - | - | - | - | - | - |

Table 1 continued

| Fungal genera and species | Luncheon | | | Minced meat | | | Livers of poultry | | | livers of cattle | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ATC | N.C.I | OR | ATC | N.C.I | OR | ATC | N.C.I | OR | ATC | N.C.I | OR |
| *Neurospora crassa* | 100 | 2 | L | - | - | - | - | - | - | 40 | 1 | R |
| *Penicillium* | 280 | 3 | L | 280 | 4 | M | 260 | 3 | L | 280 | 3 | L |
| *P.chrysogenum* | 60 | 1 | R | 160 | 2 | L | 180 | 2 | L | - | - | - |
| *P.corylophilum* | 40 | 1 | R | - | - | - | - | - | - | - | - | - |
| *P.funiculosum* | - | - | - | - | - | - | 80 | 1 | R | - | - | - |
| *P. viridicatum* | 180 | 2 | L | 120 | 2 | L | - | - | - | 280 | 3 | L |
| *Scopulariopsis* | 140 | 2 | L | - | - | - | 60 | 1 | R | - | - | - |
| *S. brevicalis* | 140 | 2 | L | - | - | - | - | - | - | - | - | - |
| *S.Koningii* | - | - | - | - | - | - | 60 | 1 | R | - | - | - |
| Gross total count | 2680 | | | 7340 | | | 7460 | | | 4760 | | |

**ATC : Average total count (per gm fresh weight)**

**N.C.I. : Number of cases of isolation**

**OR : Occurrence remarks.**

**H : High occurrence, from 6 to 10 cases**

**M:Moderate occurrence, from 4 to 5 cases.**

**L:Low occurrence from 2 -3 cases**

**R: Rare occurrence, one case only.**

**Table 2. Natural occurrence of mycotoxins in luncheon, minced meat, livers of poultry and imported bulls.**

| Types of samples | positive samples out of 10 | Mycotoxin detected | Mycotoxin concentration (µg/kg) | Mean concentration* (µg/kg) |
|---|---|---|---|---|
| Luncheon | 4 | Aflatoxins($B_1$, $B_2$, $G_1$and $G_2$) | 11, 22, 27 | 20 |
| | | Ochratoxin A | 95, 105 | 100 |
| minced meat | 4 | Aflatoxins($B_1$, $B_2$, $G_1$and $G_2$) | 18 , 22, 29 | 23 |
| | | Citrinin | 75, 93, 110, 135 | 103 |
| | | Sterigmatocystin | 115 | 115 |
| livers of poultry | 5 | Aflatoxins($B_1$, $B_2$, $G_1$and $G_2$) | 29, 43 | 36 |
| | | Citrinin | 65, 77, 125 , 165 | 108 |
| livers of imported bulls | 5 | Aflatoxins($B_1$, $B_2$, $G_1$and $G_2$) | 26 , 38 , 65 , 80 | 54 |
| | | Ochratoxin A | 145 | 145 |

* Each value represents the mean of positive samples