MYCOLOGICAL QUALITY AND AFLATOXIN RESIDUES IN SOME POULTRY MEAT IN DAMANHOUR CITY

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	ABSTRACT
Received at: 26/12/2013	A total number of 60 frozen poultry meat samples (20 of each of chicken, duck and quail carcasses) were randomly collected from various meat markets at Damanhour city for mycological examination and aflatoxin residues detection. The results revealed that the mould counts (mean \pm SE) in chicken, duck and quail meats were
Accepted: 16/2/2014	2.0x10 ³ ± 4.6x10 ² ; 2.3x10 ³ ± $6.2x10^2$; 7.4x10 ² ± 1.6x10 ² mould/g, respectively. Whilst yeast counts were $3.2x10^4 \pm 7.0x10^4$; $1.4x10^4 \pm 3.1x10^3; 4.5x10^3 \pm 1.6x 10^3$ cfu/g. The most common fungi belonged to genera Aspergilli (A. flavus and A. niger), Pencillum, Fusarium, Mucor, Cladosporium, Nigrospora, Curivlaria, Geotricum, Aurebasida and Drechlera, while three yeast genera were detected: Candida (C.albicans, C.tropicals), Rodotroulla and Cryptococcus. Toxicity screening proved that two isolates (25%) out of eight Asperigillus fungi were toxigenic. ELISA quantification of aflatoxin revealed that AFB ₁ was found in four out of the five detected chicken meat samples (20, 20,10 and 16 µg/kg), while the 5th one has 0.4 µg/kg aflatoxin B ₂ . Two of duck meat samples were positive to aflatoxins. The first one has 0.5 µg/kg of aflatoxin G ₂ , the second, however, has 8 µg/kg aflatoxin B ₁ . Also two of quail meat samples were have aflatoxin residues (10%), the first one has 15 µg/kg Aflatoxin B ₁ and the second has 10 µg/kg Aflatoxin G ₁ and 0.25 µg/kg Aflatoxin G ₂ . The present study warrants about the need for strict safety measures to coupe the potential hazards via the use of poultry meat as an indispensible protein source that carries with cumulative factors of hepatic failure in a country with a long history of endemic hepatitis.

Key words: Poultry meat, Mycological quality, Aspergillus flavus, Aflatoxin.

INTRODUCTION

Fungi are widely distributed in nature and affect man and animal through various ways due to lack of hygienic measures during handling, processing, transportation and storage of food. Fungi pollute meat and other animal tissues at the time of slaughter as a result of contaminated environment and factors which favour growth of fungi and mycotoxins production as high temperature, water activity or relative humidity, carbon dioxide, pH, oxidation potential and unclean animal houses (Jay, 1978).

Mycosis and allergies due to fungal infection are probably a major concern in the consumption of meat and there is a potential for mycotoxins to enter meat, either by direct fungal growth on meat consumed by human or by indirect carry over from animal feed to edible tissues. Also indirect way may result from consumption of animal product as meat, which contains residues of mycotoxins resulting from feeding mouldy feed to food producing animal (Bullerman, 1979 and Pestka, 1986). The contamination of meat with mould leads to either food poisoning or spoilage and render it to be of inferior quality (Wafia and Hassan, 2001). Also, the consumption of the contaminated meat by mould and their mycotoxins induces haemorrhages with hepatotoxic, nephrotoxic, neurotoxic, dermatotoxic, genotoxic, teratotoxic, carcinogenic or hormonal effects and immunosuppression (Cheo, 1991 and Hassan et al., 2004).

The serious role played by fungi due to either the economic loss or public health hazard caused the advanced countries to carry out mould and yeast count as a standard test for checking the general sanitary hygiene conditions during meat processing (Foster et al., 1958). Moreover, meat spoilage due to fungal contamination may be caused by enzymatic activities of some strains. Frazier and Westhoff (1978) mentioned that moulds and yeasts are responsible for contamination of meat and its products, which may lead to food poisoning and/or spoilage including stickness, whiskers, black spots,

green spots or patches, fat decomposition and off odour. Koburger and Jacer (1987) mentioned that 31% of the examined fungi showed proteolytic properties, while 11% showed lipolytic properties.

Aflatoxins are toxic, carcinogenic secondary metabolites produced by certain strains of the genus *Aspergillus* (A.flavus and A.parasiticus). There have been many observations of aflatoxin residues in the tissues and eggs of broiler chickens and layers hens (Rodricks and Stoloff, 1997), but little is known about aflatoxin residues in ducks and quails. These metabolites are recognized as food and feed contaminants of great economic importance throughout the world. The widespread distribution of the aspergilli in nature suggests the potential hazard of human and animal intoxication.

All aflatoxins are chronically toxic to varying degrees. Alfatoxin B1 is considered to be among the most potent carcinogens known and has been linked epidemiologically with cases of human liver cancers in a number of developing countries (Aikens and Norman, 1998).

Several studies have demonstrated that very young children may be exposed to aflatoxins even before they are weaned because mothers consuming aflatoxin in their food, may secrete aflatoxin M_1 in their milk. There is no doubt about the potential danger of aflatoxin in food and every effort should be made to reduce or, if possible, eliminate contamination.

Therefore, the aim of the present study was to study the fungal contaminations of poultry meats, screening the isolated fungi for aflatoxins production as well as monitoring these types meats for aflatoxins residues.

MATERIALS and METHODS

A total of 60 frozen poultry meat samples (20 of each of chicken, ducks and quails carcasses) were randomly collected from meat markets at Damanhour city in Al-Behera governorate. Each carcass sample was wrapped separately in a sterile polyethylene bag under complete aseptic conditions and transferred directly to the laboratory without delay in an ice box. In the laboratory, the whole surface of each sample was aseptically exposed and about 75g of mixed breast and thigh muscles meat from each were aseptically minced for moulds and yeast culturing and aflatoxins extractions. Samples were kept frozen at -20°C in a laboratory refrigerator till mycological examination.

I-Mycological examination:

1. Preparation of samples (ICMSF, 1978)

Ten grams of frozen poultry meat samples were thawed and transferred to a sterilized homogenizer

flask containing 90 ml of 0.1% sterile peptone water. The contents were homogenized at 14000 r.p.m. for 2.5 minutes to provide a dilution of 10^{-1} . The homogenate was allowed to stand for 5 minutes at room temperature, then 1 ml of homogenate was transferred with a sterile pipette into a sterile test tube containing 9 ml of 0.1% sterile peptone water to obtain a dilution of 10^{-2} . Then further decimal ten fold serial dilutions up to 10^{-4} were prepared.

2. Total mould and yeast counts (Baily and Scott, 1978):

The total mould and yeast counts was done by using Sabourad's dextrose agar medium, supplemented with chloramphenicol and chlortetracycline (100 mg of each) as described by koburger (1970).

One ml from the previously prepared serial dilution was aseptically transferred into sterile Petri dishes then about 15ml of the sterile Sabourad's dextrose agar previously melted and cooled at 45°C were added and mixed thoroughly in a horizontal position. After solidification, inoculated as well as control agar plates were incubated in an inverted position at 25°C for 7 days.

Mould as well as yeast were counted separately and recorded as total mould and yeast count/g of meat. The isolated fungi were identified individually by macro- and microscopic characteristics according to Samson (1979), Pitt and Hoching (2009), while yeast isolates were identified according to Kriger Van Rij (1984) and Tibor and Larry (1996).

3. Screening of the aflatoxin-producing Aspergilli: Production of aflatoxins by isolated strains of moulds were determined by the screening method used by De Vogel *et al.* (1965). Fluorescence under longwave length ultraviolet light was checked after 3 and 10 days of 27° C. Isolates which showed blue or green fluorescence by the screening test were inoculated into 50 ml of YES broth (20%sucrose and 2%yeast extract). Inoculated broth cultures were incubated at 27° C for 7 to12 days. After that, the mould cultures were extracted with twice by shaking for 10 minutes with 75 ml of chloroform, the chloroform layer was collected with a separatory funnel, filtered, and evaporated to dryness at 40° C with a flash evaporator (Strzelecki *et al.*, 1969).

Production of aflatoxin was determined visually by comparing the sample with aflatoxin standards (Southern Utilization Research and Development Laboratories, New Orleans, La.) under ultraviolet rays (256 nm and 365 nm). From producing stains, the chloroform extract was chromatographed on a preparative scale, and the suspect spot removed. The aflatoxin was eluted from the silica gel with chloroform and filtered, and the ultraviolet absorption spectra were obtained by a Perkin-Elmer model 202 spectrophotometer.

The concentration of the aflatoxin in the sample $(\mu g/Liter)$ is calculated by the formula:

$$\frac{S \times Y \times V}{W \times Z}$$

S = Volume of aflatoxin standard, in µl, of equivalent intensity to Z µl of sample

 \mathbf{Y} = Concentration of aflatoxin Standard in μ g/ml

 $\mathbf{V} =$ Volume of solvent, in μ l, required to dilute final extract

Z = Volume of sample extract, in µl,required to give fluorescence intensity comparable to that of S µl of the standard.

W =Millilitrs of sample applied to column.

II- Aflatoxin determination by ELISA technique (Ilie *et al.*, 2007):

Using aflatoxins quantitative test kit (Veratox, Neogen Corporation) and Behring E 131 15 Enzyme-Linked Immunosorbent Assay, Auto reader.

- Preparation of sample extract:

1- 50 gms of each poultry meat sample were blended with 250 ml of methanol 70% / water solution for 2

minutes in a high speed blender (sterilimixer Lab. Pbi.International Milano Italy, 16500 giri /min.).

2- The extract was filtrated by pouring at least 5-15 ml through a Whatman No 1 filter paper and the filtrate was collected as a sample.

-Detection of aflatoxins residues: according to manufacturer protocols.

- Estimation of aflatoxins residues:

- The colors of the resulting solutions for each of aflatoxins (B1, B2, G1 and G2) were observed. Blue color indicates negative samples, while red color indicates a strong positive.

- Wipe bottom of microwells and read in a micro-well reader blanked on air using a 650 nm filter.

- The concentration of aflatoxins in the samples are estimated from curves relating absorbance to the concentration of the aflatoxins standards.

RESULTS

Table 1: Statistical analytical results of mycological examination of poultry meat samples (n=20 in each group).

		Mould Count				Yeast Count					
Type of samples	Positive Samples		Mean	S.E.M.	Positive Samples		Mean	S.E.M.			
	No.	%	-	-	No.	%	-				
Chicken meat	19	95	2.0 X10 ³	4.6 X 10 ²	19	95	3.2 X 10 ⁴	7.0 X 10 ³			
Duck meat	15	75	2.3 X 10 ³	6.2 X 10 ²	18	90	1.4 X 10 ⁴	3.1 X 10 ³			
Quail meat	16	80	7.4 X 10 ²	1.6 X 10 ²	17	85	4.5 X 10 ³	1.6 X 10 ³			

No. =Number S.E.M.= Standard error of mean.

Table 2: Incidences of isolated mould genera from examined samples.

Type of meat	Chie	Dı	ıck	Quail		
Isolated genera	No.	%	No.	%	No.	%
Aspergillus flavus	1	5	2	10	5	25
Aspergillus niger	0	0	1	5	1	5
Pencillium sp.	3	15	0	0	9	45
Fusarium sp.	0	0	0	0	2	10
Mucor sp.	16	80	11	55	6	30
Cladosporium sp.	2	10	0	0	3	15
Nigrospora sp.	0	0	1	5	1	5
Curivlaria sp.	0	0	0	0	2	10
Geotricum sp.	0	0	1	5	1	5
Aurebasida sp.	0	0	1	5	0	0
Drechlera sp.	1	5	0	0	0	0

Type of meat	Chi	Chicken		ıck	Quail		
Isolated genera	No.	%	No.	%	No.	%	
Candida albicans	19	95	11	55	16	80	
Candida tropicalis	4	20	1	5	3	15	
Rhodotroula sp.	5	25	18	90	3	15	
Cryptoccus sp.	3	15	5	25	0	0	

Table 3: Incidences of isolated yeast genera from examined samples.

Table 4: Incidences of mycotoxigenic Aspergillius flavus contaminated poultry meats.

	Total isolates of Aspergillus flavus	Mycotoxigenic strains of isolated Aspergillus flavus						
	-	Incidence Levels of aflatoxin re (µg/L)					esidues	
	-	No.	%	B ₁	B ₂	G ₁	G ₂	
Chicken	1	0	0	0	0	0	0	
Duck	2	0	0	0	0	0	0	
Quail	5	2	40 -	40	0	0	0	
	5		40 -	0	0	10	0.01	

Table 5: Aflatoxin residues levels in examined poultry meat samples (n=20 of each).

Aflatoxins	Incidences		Detected Samples							
	Incluences	Sampla	Levels (µg/kg)							
Type of meats	No. of detected samples	%	- Sample no.	B ₁	B ₂	G ₁	G ₂	Total		
	5	25	2	20	0	0	0	20		
			8	20	0	0	0	20		
Chicken			9	0	0.4	0	0	0.4		
			10	10	0	0	0	10		
			18	16	0	0	0	16		
Duck	2	10	3	0	0	0	0.5	0.5		
Duck			5	8	0	0	0	8		
Quail	2	10	6	15	0	0	0	15		
			7	0	0	10	0.25	10.25		

N.B.: µg/kg=ppb

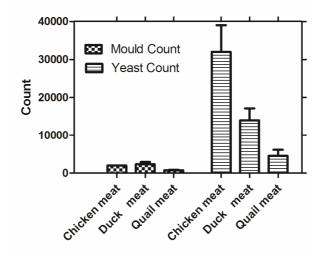


Fig. 1: incidence of total mould and yeast counts in different poultry meat samples:

There is marked difference (p < 0.001) in the intensity of mould count in comparison with that of yeast in poultry meat samples. The intensity of yeast in poultry meat samples showed extreme significant difference between different sample origins (p < 0.01) with higher level recorded in chicken meat samples.

DISCUSSION

Mycological examination:

Table (1) and Fig.(1) were showed that the means of mould counts in chicken ,duck and quail meat samples were 2.0 $\times 10^3 \pm 4.6 \times 10^2$, 2.3 $\times 10^3 \pm 6.2 \times 10^2$ and 7.4 $\times 10^2 \pm 1.6 \times 10^2$ moulds/g with incidences of 95%, 75% and 80%, respectively. Also the means of yeast counts (cfu/g) were 3.2 $\times 10^4 \pm 7.0 \times 10^3$, 1.4 $\times 10^4 \pm 3.1 \times 10^3$ and 4.5 $\times 10^3 \pm 1.6 \times 10^3$, respectively.

The low number of fungi in examined samples may be as a result of freezing of carcesses after processing as stated by Khater *et al.* (2012) who recorded an increase in their numbers as a result of cutting and deboning processes.

The data tabulated in Table (2) were showed that the *Aspergillus flavus* and *A. niger* were identified in chicken, duck and quail meats by the following percentages (5 and 0), (10 and 5) and (25 and 5); respect., *Pencillium sp.* (15, 0 and 45) and *Fusarium sp.* (0, 0 and 10). The fore-mentioned moulds are of great importance due to potential of mycotoxins production. While other mould genera could be isolated by different percentages as follow: *Mucor sp.* (80, 55 and 30), *Cladosporum sp.* (10, 0 and 15), *Nigrospora sp.* (0, 5 and 5), Curivlaria sp. *Curivlaria sp.* (0, 0 and 10), *Geotricum sp.* (0, 5 and 5), *Aurebasida sp.* (0, 5 and 0) and Drechlera *sp.* (5, 0 and 0), respectively.

Most of the these mould genera were previously isolated from chicken, meat and meat products in Egypt but in different frequencies (Hegazi *et al.*, 1992; Khater *et al.*, 2012). Lowry and Gill (1984) stated that the air is considered the main source of contamination of meat with mould spores.

The most frequently isolated yeast genera form the examined samples were Candida albicans (95%, 55% and 80%), Candida tropicalis (20%, 5% and 15%), Rhodotroula sp. (25%, 90% and 15%) and Cryptococcus sp. (15%, 25% and 0%), respectively, (table, 3).

The ability of the mould and yeast species to grow at low temperatures and their proteolytic and lipolytic activities may cause spoilage of poultry meats by breaking down their components and liberation of different acids and gases with subsequent change of their odour and flavour. Moreover, mould growth on meat causes economic losses from discolouration, poor appearance and off flavours, in addition, some moulds are capable of producing toxic metabolites mycotoxins such as aflatoxins which are carcinogenic (Pitt and Hoching, 2009).

From the public health of view, Aspergillus and Mucor species were incriminated in pulmonary aspergillosis, pulmonary allergy, skin infections, sinusitis and otomycosis and deep wound infections for meat handlers (Banwart, 1989; Jawetz *et al.*, 1974). It has been stated that some species of Pencillium were found to be associated with pulmonary and urinary tract infections as well as yellow "rice disease" causing several deaths in man (Washington, 1981). Cladosporum species may induce chromatomycosis and brain abscesses (Edris, 1986; Jawetz *et al.*, 1974). Mucor and Cladosporum species may cause mycotic abortion

and allergy in animals and humans (Khosravi *et al.*, 2008). Fusarium species may cause mycotic keratitis and skin infections (Banwart, 1989; Jawetz *et al.*, 1974).

The results in Table (4) showed that chicken and duck meats were the least contaminated poultry meats by Aspergilli (5% and 10%) which were found non toxigenic, but quail meats were moderately contaminated (25%) by A.flavus and 40% of isolated strains were aflatoxigenic. The type of aflatoxins secreated in broth culture by A.flavus were B₁,G₁ and G₂ and their concentrations were 40, 10 and 0.01 μ g/liter. This result was in agreement with Abalako and Oloyede (1983) and Shaltout (1996) who reported that A.flavus isolated from meat products could secrete aflatoxins B1, B2, G1 and G2 in yeast extract sucrose (YES) broth medium.

Mould and yeast contaminations of poultry meat indicated improper sanitary and health conditions during handling, processing and storage.

Aflatoxicological examination:

Mycotoxins contaminations of food and feeds remains a global problem .The United Nations of Food and Agriculture Organization has estimated that as much as 25% of world food is significantly contaminated (Smith *et al.*, 1994).

The results in Table (5) showed that chicken meats were exhibited the highest incidence of aflatoxins residues (25%) than those of duck and quail (10% of each). AFB₁ was detected in 4 chicken meat samples (20%) at concentrations ranged from 10 to 20 $\mu g/kg$, but AFB₂ was found only in one meat sample (5%), at a concentration of 0.4 μ g/kg. While AFG₁and AFG₂ were not detected. These high aflatoxins levels in chicken meat may be due to that the A.flavus appears to be the most important causal agent of contamination of maize which is the basic constituent of poultry feeds (Atehnkeng et al., 2008). In duck meats, AFB1 and AFG2 was detected in only one sample of each (5%) at concentrations of 8 μ g/kg and $0.5 \mu g/kg$, respectively. While quail meat samples were the highest contaminated with alfatoxigenic A.flavus (40% of isolated strains), there was a detection of only one sample (5%) contained AFB1 residues at a concentration of 15 µg/kg and other one was contained both AFG1 and AFG2 at concentrations of 10 and 0.25 µg/kg, respectively. Therefore, the presence of toxogenic moulds in any food does not automatically means the presence of mycotoxins residues, but rather than a potential for mycotoxin contamination existence. It seems there is no relationship between the presence of toxigenic strains of A. flavus and aflatoxin contamination of meat samples (Ismail and Zaky, 1999). According to Leeson et al. (1995) residues of aflatoxins can be found in poultry meat and products, but results of a withdrawal trial showed that poultry could metabolize

and eliminate aflatoxin from their tissues in a relatively short time period (72-96 h).

The results of the total detected alfatoxins were not exceeded the maximum permissible limit. Food and Drug Administration (FDA) established regulatory working guidelines on the acceptable levels of acceptable levels of aflatoxins in human foods set at 20 ppb for total aflatoxins (FAO, 2004), with the exception of milk which has an action level of 0.5ppb of aflatoxins (Bullerman, 1979). Also, some of these recorded results were exceeded WHO permissible limit, 15 ppb (Jelinek et al., 1989). The regulatory limit for AFB1 in many countries is 5 ppb (Gilbert, 1991; Van Egmond, 1991). It has to be recognized that these agencies faced a hard dilemma when setting these limits and this is reflected in the observation that clearly the group would have preferred a lower figure but felt that the danger of malnutrition was greater than the danger that aflatoxin would produce liver cancer in man. The concentrations of the determined aflatoxins were significant when compared to the African maximum permissible limits of 5 µg/kg and 20 µg/kg for AFB1 and total aflatoxins in foods respectively (FAO, 2010). The overall impact of mycotoxins on health however is dependent on the concentrations and duration of exposure, the toxicity of the compound, the body weight of the individual, the synergistic effects of mycotoxins, environmental factors and other effects (Kuiper-Goodman, 1991; Bennett and Klich, 2003).

Mohamed (2005) stated that all chicken meat samples were contaminated with AFB_1 , B_2 , G_1 and G_2 with mean values of 4.27 ± 0.85 , 4.5 ± 0.29 , 4.79 ± 0.43 and 4.69 ± 0.42 ppb, respectively. Also, Bintvihok and Kositcharoenkul (2006) detected AFB₁ in 30 (11.11%) of broiler muscle with mean value of 0.02 ± 0.01 ppb. Maikanov (1986) stated that pectoral muscle of ducks were contaminated with AFs (1.8 µg/kg). Also, Teleb and Fakhry (1988) determined AFB_1 in breast muscle at concentrations from 8.03-18.61 ppb. Higher results was recorded by Mahmoud et al. (2001) who stated that in the citrinin contaminated examined samples, aflatoxins were also detected in 3 and 2 samples of minced meat and livers of poultry with means of 23 and 36 µg/kg. They added that aflatoxins (B1, B2, G1 and G2) were detected in 4 samples of livers from imported bulls with a mean of 54 µg/kg. Hassan (1995) detected AFG₂ (80 μ g/kg) in one (4%) of table blady eggs. A review by Blank (2002) showed that the carryover of mycotoxins into edible tissues is relatively low and is dependent on the specific mycotoxins and animal species. For every 1200 parts of aflatoxin intake, 1 part is deposited in meat and for every 2200 parts, 1 part is transferred into eggs (Coker et al., 1984).

The AFB1 and AFG1 detected in samples are proven human carcinogens and are thus classified as Group1 carcinogens with AFB2 as Group 2B

probable human carcinogens (*IARC*' International Agency for Research on Cancer ', *1993*). Documented evidence have shown that AFB1 exposure exacerbates protein calori malnutrition, thereby suppressing growth as well as immunoglobulin (IgA) response to some vaccine challenges, among the African children (Gong *et al.*, 2004; Turner *et al.*, 2003). Consequently the resultant effects of AFB1 exposure may include growth and immune function suppression.

For prevention and reduction of mycotoxins hazards on human health, it is necessary to create both global and national strategies for combating mycotoxins, advanced diagnostic techniques and procedures and control programes should be initiated in both agricultural and veterinary aspects. The first should take care of the crops as ingredients for animal feedstuffs during growth and at and postharvesting, and also the veterinary authorities should take care of the control of animal health and also animal products as eggs, milk, meat and other edible tissues.

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مدى تواجد الفطريات وبقايا سموم الأفلاتوكسين في لحوم بعض انواع الطيور في مدينة دمنهور

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