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MYCOFLORA AND NATURAL OCCURRENCE OF MYCOTOXINS OF CHICKEN STOCK

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الفلورا الفطرية والتواجد الطبيعي للسموم الفطرية في مرقة الدجاج

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الهدف من الدراسة التعرف على الفلورا الفطرية والسموم الفطرية المتواجدة طبيعيا في ٤٥ عينة من مرقة الدجاج بمصر .

تم عزل وتعريف ٤٢ نوعاً فطرياً بالإضافة إلى صنفين تنتمي إلى ٧ أجناس وذلك على الوسط الغذائي جلكوز كزابكس أجار ، كزابكس أجار المزود بتركيز ١٠٪ كلوريد صوديوم، وكانت أكثر الأنواع أنتشاراً اسبرجيلس (١٠ أنواع) ايروتيم (٦ أنواع) والبنسليوم (٦ أنواع) على الوسطين الغذائيين .

واشتملت الدراسة امكانية تواجد سموم الافلاتوكسين والزيرالينون والزيرالينول طبيعيا وبإستخدام طرق التحليل الكروماتوجرافي (TLC) وجد أن ١٩ عينة ملوثة بسموم الأفلاتوكسين G_2 , G_1 , B_2 , B_1 والزيرالينون والزيرالينول .

Key Words: Mycoflora, Mycotoxins, Chicken Stock.

ABSTRACT

Twenty four species and one species variety (belonging to 11 genera) and 20 species with the addition of two species varieties (belonging to 7 genera) were isolated on glucose-Czapek's agar and glucose-Czapek's agar medium fortified by 10% sodium chloride respectively, from 45 samples of manufactured chicken stock collected from Egypt. *Aspergillus* (10 species), *Eurotium* (6 species) *Penicillium* (6 species) and *Emericella* (one species) were the most common genera isolated from the two different media. Thin layer chromatographic analysis revealed that 19 samples were contaminated by aflatoxin zearalenone and/ or zearalenol.

INTRODUCTION

Fungi can be important in spoilage of meat and meat products, leading to great economic losses. They also may constitute a potential public health hazard by production of a wide variety of mycotoxins. Some fungi are pathogenic to man, and the non pathogenic species may impart a moldy odour and taste. Fungi may also promote rancidity of fat (Gracey, 1981). Fungi contaminate meat and its products in several ways. The contamination by fungi may be either in living animals, before processing or during transportation and storage. Hence, the study of occurrence, composition and numbers of various fungi in chicken stocks is of potential importance and significance.

MATERIALS AND METHODS

Isolation and Identification of Fungi

The dilution-plate method as described by Christensen (1963) and employed by Moubasher *et al.* (1979) was used for the estimation of fungi in chicken stock. Glucose-Czapek's agar and Glucose-Czapek's agar fortified by 10 % sodium chloride were used for isolation of glucophilic and halophilic (or halotolerant) fungi respectively. Rose bengal (1/15000) mixed with streptomycin (1/30000) (Smith and Dawson, 1944) were used as bacteriostatic agents. Five plates were used for each sample. The plates were incubated at 28°C for 8-15 days during which the developing fungi were counted and identified. The colonies of slow growing fungi as well as mycelial bits were transferred to slants or plates containing 1%

glucose- Czapek's agar + 0.05 yeast extract; malt extract; potato-dextrose or corn meal agar medium to ensure precise counting and identification.

Mycotoxin extraction and thin layer chromatographic analysis

25 gm of each chicken stock sample was defatted by extraction with n-hexane for 10h using a Soxhlet-type extractor. The defatted residue was extracted for another 10h with chloroform. The chloroform extract was dried over anhydrous sodium sulphate, filtered then distilled to near dryness under vacuum or a stream of nitrogen. The residue was diluted with chloroform to 1 ml.

A known volume (0.05 ml) of the chloroform extract was applied to pre-coated silica gel plate type 60, F254 TLC (MERCK). Five microliters of chloroform containing about 5µm of mycotoxin reference standard were also applied to each plate. Standards used were: aflatoxins B1, B2, G1 & G2, citrinin, ochratoxin A, patulin, sterigmatocystin, diacetoxyscirpenol, T-2 toxin, zearalenone and zearalenol.

The plates were developed with two solvent systems; toluene-ethylacetate- formic acid (6:3:1, v/v/v) and chloroform- methanol (97:3, v/v). Developed plates were examined before and after spraying with different reagents under short wave (254nm) and long wave (365nm) ultraviolet irradiation according to the methods of Scott *et al.* (1970).

Confirmatory test and quantitative determination of the detected toxins

Aflatoxin: The chemical confirmation method for aflatoxin B1 by direct formation of hemiacetal (aflatoxin B2a) on the thin-layer plate before chromatography as described by Przybylski (1975) was used. Aflatoxin content of the toxic extract was estimated using the technique described by Coomes *et al.* (1965).

Zearalenone and zearalenol: The identification of zearalenone and zearalenol were established by different colour reactions with spray reagents (Caldwell *et al.* 1970; 1974). The procedure described by Mirocha *et al.* (1971) was used for quantitative analysis of zearalenone.

Sources of chicken stocks

Five samples of each of nine different brands of chicken stock were purchased from different markets in Assiut governorate, Egypt.

RESULTS AND DISCUSSION

Glucophilic fungi

Twenty four species and one variety of glucophilic fungi belonging to 11 genera were isolated on plates of glucose-Czapek's agar 28°C ± 2°C (Table, 1). *Aspergillus* was the most common genus and recorded from all samples comprising 96.9% of total fungi. It was represented by 9 species and one species variety of which *A. fumigatus, A. flavus* and *A. niger* were highly frequent. They occurred in 43, 40 and 35 out of 45 samples tested comprising 69.3%, 7.6% and 15.6% of total fungi respectively (Table, 1). *A. ochraceus* (11 out of 45 samples tested and 0.8% of total fungi) was encountered with moderate frequency of occurrence. These results are in harmony with those obtained by other workers. Kamel *et al.* (1976) and Rifai & Loot (1969) isolated *Aspergillus niger, A. fumigatus* and *A. flavus* as the common *Aspergillus* species from meat at slaughter houses, butcher's shops and cold stores. Ibrahim *et al.* (1983) identified 3 species of *Aspergillus* (*A. fumigatus, A. flavus* and *A. niger*) from 1440 samples of different organs of 480 dead broiler chickens. Fahmy (1986) found that Aspergillus was the most common genus in frozen chicken and isolated five species which *A. niger, A. flavus* and *A. fumigatus* were the most dominant. The remaining *Aspergillus* species were isolated with low or rare frequencies of occurrence.

Penicillium (represented by 6 species) and *Emericella* (one species) were isolated in frequently from 9 and 7 out of 45 samples tested, and comprised 0.8% and 0.4% of total fungi respectively (Table 1). In a previous study Ibrahim *et al.* (1983) isolated *Penicillium* from 12 samples out 480 samples tested of lung of dead broiler chickens. Abdel Rahman *et al.* (1984), examined the fungal flora of 60 samples of meat products and recorded that *Penicillium* was the most common fungus. Abdel Rahman *et al.* (1985) isolated 1261 strains of *Penicillium* from the surface of beef quarters, packed beef and from frozen poultry, walls, floor, roofs and air of cold stores. They found that *Penicillium* was the most dominant genus and represented by 10 species of which *P. verrucuosum* var *cyclopium, P. nigricans, P. citrinum, P. multicolor* and *P. brevicompactum* were the most common. Also Fahmy (1986) found that *Penicillium* was the most common genus and occupied second place with regard to the number of cases of isolation from frozen meat (62%) and chicken (40%).

The remaining genera (8) and species (8) were isolated rarely and accounted collectively for less than 3% of total fungi (Table 1).

Halophilic and halotolerant fungi

The results obtained on 10% NaCl-glucose-Czapek's agar were basically similar to those obtained on glucose-Czapek's agar, the following observations can be drawn. The gross total count (14885 colonies) was diminished on 10% Nacl-glucose-Czapek's agar (compared with 28710 colonies per g on glucose-Czapek's agar) Narrowest spectra of genera and species were recorded (7 genera and 20 species + 2 varieties) on 10% Nacl-glucose -Czapek's agar (compared with 11 genera and 24 species + one variety on glucose-Czapek's agar). Several genera and species were recovered on glucose-Czapek's agar and not on 10% NaCl-glucose-Czapek's agar. However, the genus *Eurotium* (represented by 5 species and one variety) was recorded only on 10% NaCl-glucose-Czapek's agar (Table, 1). Most of the *Eurotium* species recorded in this study were previously isolated from different meats and meat products (Klare, 1970; Abdel-Rahman, 1981; Abdel-Rahman *et al.* 1984; Farghaly, 1985 and Fahmy, 1986). Moubasher *et al.* (1988) classified *E. rubrum, E. repens, E. amstelodami* and *E. chevalieri* as highly halophilic fungi which grow on 5% to 20 or 25% NaCl.

Natural occurrence of Mycotoxins of the different chicken stock samples

The natural occurrence of mycotoxins in meat products has become of increasing interest because of the widespread use of these products in the world. Analysis of 45 samples of chicken stock for the natural occurring mycotoxins, showed that 6 samples were contaminated by aflatoxins B1, B2, G1 and G2 (10-25 µg/kg.) These samples were naturally contaminated by aflatoxin-producers (*A. flavus* and/or *A. parasiticus* Table, 2). Surveillance of meat products in the market place has been limited. Aflatoxins have been in liver, kidney and other tissues of pigs in feeding trials with aflatoxin-contaminated diets (Jarvis, 1976; Bartos & Matyas, 1980). Aflatoxins have been

Table 1

Average total counts (ATC, calculated per g dry weight in all samples), number of cases of isolation (NCI, out of 45 samples) and occurrence remarks (OR) of fungal genera and species isolated from chicken samples on glucose-Czapek's and 10% sodium chloride-glucose Czapek's agar media at 28°C(±1°C).

General and Species	Glucose Czapek's agar		10% NaCl Czapek's agar	
	ATC	NCI&OR	ATC	NCI&OR
Aspergillus	27550	45H	10125	43H
A. fumigatus Fresenius	19889	43H	310	5R
A. flavus Link	2190	40H	920	25H
A. niger Van Tieghem	4471	35H	1995	27H
A. ochraceus Wilhelm	240	11M	6410	23H
A. awamori Nakazawa	360	8L	90	5R
A. terreus Thom	130	7L	170	6L
A. candidus Link	80	6L	30	3R
A. sydowii (Bain and Sari) Thom & Church	110	5R	150	7L
A. flavus var columnaris Raper & Fennell	20	2R	50	4R
A. versicolor (Vull) Tiraboschi	60	2R	0	0
Eurotium	0	0	3920	45H
E. amstelodami Mangin	0	0	1560	33H
E. chevalieri Mangin	0	0	790	29H
E. montevidenses (Talic & Mackinnon)	0	0	870	23H
E. chevalieri var. intermedium (Thom & Raper) Malloch & Cain	0	0	250	12M
E. repens De Bary	0	0	220	11M
E. rubrum Konig, Spieckermann & Bremer	0	0	230	11M
Penicillium	240	9L	160	8L
P. corylophilum Dierckx	50	3R	60	4R
P. digitatum Sacc.	70	3R	80	2R
P. duclauxi Delacr.	20	2R	0	0
P. funiculosum Thom	50	2R	0	0
P. chrysogenum Thom	20	1R	20	2R
Emericella nidulans (Eidam) Vuillemin	120	7L	350	15M
Scopulariopsis brevicaulis Bain.	80	4R	10	1R
Cladosporium cladosporioides (Fresen) De Vries	40	2R	300	4R
Cochliobolus lunatus Nelson & Hassis	40	2R	0	0
Mucor hiemalis Wehmer	540	2R	0	0
Paecilomyces variotii Bainier	30	2R	20	1R
Rhizopus stolonifer Elrenb. exFr. vuil	30	2R	0	0
Botryotrichum piluliferum Saccardo & Marchal	20	1R	0	0
Trichoderma viride Pers. ex S. F. Gray	20	1R	0	0
Gross total count	28210		14885	
Number of genera	11		7	
Number of species & varieties	24+1		20+2	

* Occurrence remarks (OR)
* H = Highly occurrence : between 23-45 cases
* M = Moderate occurrence : between 11-22 cases
* L = Low occurrence : between 6-10 cases
* R = Rare occurrence : between 0-5 cases

Table 2

Number of toxic samples (out of 5 tested), toxins identified, and dominant species isolated.

Sample No.	No. of toxic samples out of 5 tested	Toxins identified	Dominant species isolated
Chicken Stock Brand 1	2	Aflatoxins B_1, B_2, G_1 & G_2	**A. flavus* , ***E. amstelodami*
Chicken Stock Brand 2	1	Zearalenone	*A. fumigatus* , *E. chevalieri*
Chicken Stock Brand 3	2	Zearalenone & Zearalenol	*A. fumigatus* , *A. flavus*
Chicken Stock Instant	5	Zearalenone & Zearalenol	*A. fumigatus* , *E. amstelodami*
Chicken Bouillon brand 1	1	Zearalenone	*A. fumigatus* , *A. niger*
Chicken Bouillon brand 2	0	-ve	*A. ochraceus*, *A. niger*
Chicken Lentil Soup	2	Zearalenone & Aflatoxins B_1, B_2, G_1 & G_2	**A. flavus* , ***E. amstelodami*
Cream of Chicken Soup	3	Zearalenone	*A. fumigatus* , *A. niger*
Tomato Stock	5	Zearalenone & Zearalenol	*A. flavus* , *E. chevalieri*

** A. = Aspergillus ** E. = Eurotium*

also found in the tissues of broiler chickens and eggs of laying hens given experimentally contaminated feed (Purchase, 1972; Gelda & Layt, 1977, Girgis *et al.*, 1977; Kiermeier *et al.*, 1977; Pensalo, *et al.* 1977, Stoloff, 1977, Stoloff and Truckses, 1978 and Bullerman, 1979).

Zearalenone (120-380µg/kg) and/or zearalenol (50-170 µg/kg) were detected in the extract of 13 samples out of 45 samples of chicken stock examined. Dilution plate method (Table 2) indicated that there is no *Fusarium* sp. associated with the chicken stock contaminated by zearalenone and zearalenol, these results, in general, agree with Bullerman (1986), who stated that "the absence of toxinogenic moulds does not guarantee that the commodity is free of mycotoxins since the toxins may persist long after the moulds have disappeared". According to the literatures, this is the first report about the natural contamination of meat or chicken products by zearalenone and zearalenol. detection of zearalenone and zearalenol could be attributed to either feeding of zearalenone contaminated feed or addition of zearalenone and its derivatives to the feed of chickens. Zearalenone (RAL) was shown to be an effective growth promoting material in certain test with lambs (Andrews & Stob, 1965) and zearalenol (RAL grow) was also used as a feed additive of growing beet cattle to increase the rate of gain and feed conversion (Mirocha *et al.*, 1971).

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