

## MYCOLOGICAL EVALUATION OF POULTRY FEEDS, CONCENTRATES AND FEED INGREDIENTS

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#### ABSTRACT:

A mycological evaluation of total eighty two samples of apparently normal poultry rations (22),concentrated mixtures (18) and feed ingredients (42) were performed. The samples were collected from some poultry processing plants in different localities at Assiut Governorate, Egypt. The moisture content of the examined samples were moderately low with mean values ranged from 4.58±0.72 to 7.06±0.17. The mean values of the total mould and yeast colony count/g were varied from 1.9x10<sup>4</sup> to 5.2x10<sup>4</sup>. The broiler concentrate mixtures showed the minimum count (4.4x10<sup>3</sup>/g) while the maximum count was observed in broiler rations (1.3x10<sup>5</sup>/g). Twenty fungal genera represented by 42 species and 1 species variety were identified in the present investigation. The most prevalent fungal species were found to belong to Aspergillus followed by Penicillium. Moreover, many fungal species of less frequency distributions were also detected including Cladosporium, Gibberella, Paeciliomyces, Emericella, Hypomyces, Mucor species and others. The hygienic significance of the fungal isolates and the restrictive measures that must be considered were discussed.

#### **INTRODUCTION:**

Most significant researches on fungi have been conducted in the last decade, although the older literature revealed many instances where mouldy feedstuffs caused toxic results, altered nutrient values, or reduced the palatability. In 1959, at least 100,000 turkey poults and ducklings died in England as a result of feeding contaminated peanut meal

imported from Brazil (Purchase, 1971). The U.S. broiler industry showed sporadic occurrence over the years of haemorrhagic syndrome suspected to be due to Alternaria, Aspergillus clavatus, A. flavus and Penicillium purpurogenum (Schaible, 1970). All these events changed the attitude towards moulds in foods and feeds.

Recently special attention was paid to provide information on the incidence and activities of fungi in the mixed feeds. The poultry feeds have not received special mycological attention, in spite of the fact that they are prepared basically from the raw plant materials (Corn, Soybean, Wheat bran, etc.) and concentrate mixtures, where fungi are the most important contaminant microorganisms ( Lagrandeur & Poisson, 1968; Lovett et al., 1971; Hesseltine et al., 1976; Muntanola-Cvetkovic & Borisavljevic, 1979; Ogundero, 1980; Clarke & Hill, 1981; McGimpsey & Malone, 1981; Tabib et al., 1981; El-Kady et al., 1982. Moreno-Rome & Fernandez, 1986: Ranjan & Sinha, 1991; Chang- Yen et al., 1992 and Abarca et al., 1994). These microorganisms may be present in the final product or originate directly or indirectly from cross contamination. The unfavourable conditions of storage as high temperature and humidity were found to stimulate the fungal growth in the poultry feedstuffs (Abdel-Fattah et al.,1979; Ogundero, 1980; Bartov et al., 1982 and Ogundero 1987).

The mycological study of poultry feeds and feed ingredients are of significant importance for determining the distribution of the

mycotoxin producing fungi as their toxic metabolites have been detected in many feed stuffs. In this sense, poultry feeds are the first stage of the chain that may carry mycotoxins to man. The present work was planned to throw light on the mycological status of commercial poultry mixed feeds and their ingredients. as well as their fungal contamination and to arise the public health awareness of such contamination on human health through poultry, as well as rescuing the birds from such contamination. Moreover, special attention was paid to the mycotoxin producing fungi, which will be reported elsewhere.

#### **MATERIALS & METHODS:**

### A- Collection of the samples:

Eighty two samples of poultry feeds were collected from some poultry processing plants in different localities at Assiut Governorate (Beni-Mor, Arab El-Awamer, Dronka, Rifa and Manfalout). Out of 82 samples collected, 22 were poultry rations(final product), 18 concentrate mixtures and 42 were feed ingredients. The samples were collected in sterile polyethylene bags and transported to the laboratory for their mycological analysis.

## B- Determination of the moisture content:

The moisture content of the poultry feeds was determined according to Tabib et al., (1981) and Swelim et al., (1994) by the oven method. Replicates of the samples of 10 gm for each were dried at 105 °C in an electric oven

to a constant weight. The moisture content was calculated as percentage on oven dry basis (Tabib et al., 1981 and Swelim et al., 1994).

#### C-Enumeration and identification of fungi:

The dilution-plate method described by Johnson & Curl (1972) was used for the enumeration of fungi. Dilutions were prepared by shaking 10 gm of each sample in 90 ml sterile saline solution (0.88 w/v NaCl). Serial tenfold dilutions were prepared and 1 ml aliquots of the appropriate dilution were transferred into sterile petri dishes. Dicloran rose bengal medium of King et al., (1979) was used for the isolation of fungi. Five replicates for each sample were incubated at 25 °C for 7-15 days.

The growing fungal colonies were enumerated and identified based on their macro- and microscopic characteristics using the methods of Raper & Fennel (1965), Ellis (1971)& (1976); Pitt (1979); Domsch et al., (1980); Sivanesan (1984)& (1987), Kozakiewicz (1989) and Moubasher (1993).

#### **RESULTS & DISCUSSION:**

Poultry mixed feeds and their ingredients are naturally contaminated by different species of fungi, that is mainly due to their exposure to the surrounding environment and storage under humid conditions (Beer, 1969).

In general the moisture content in such feeds is considerd as the most important factor

contributing to their moulding. From table (1), a moderate moisture content was observed in the examined samples. The highest moisture content was detected in the white corn (7.06±0.17) while the lowest was observed in the yellow corn (4.58±0.72).

Data in table (2) revealed that the highest mean of total fungal count /g was noted in the yellow corn (5.2x104) followed by laying rations (4.4x10<sup>4</sup>), broiler rations and white  $(4.1 \times 10^4)$ , wheat bran  $(3.7x10^4)$ , concentrate mixture of broiler (3.3x104), soybean meal (2.2x104). The lowest mean count was recorded in the concentrate mixture for layers (1.9x104). Concerning the total mould count/g, it easily demonstrated that the highest count was observed in the yellow corn and laying rations (table 2). This could be attributed to the high starch content (Shotwell et al., 1966) and/or high moisture content, beside the improper handling and storage specially in case of yellow corn (Bartov et al., 1982 and Ogundero, 1987). The high humidity enhances the fungal growth which is typical for the tropical and subtropical regions.

The obtained results revealed that the mean fungal count/g were ranged from  $1.9 \times 10^4$  to  $5.2 \times 10^4$ . The maximum count was found in both broiler rations and yellow corn  $(1.3 \times 10^5)$  and a minimum count in the broiler concentrate mixture  $(4.4 \times 10^3)$ . The obtained results are more or less coincident with fungal densities  $(7 \times 10^2 - 3.2 \times 10^5/g)$  obtained by Lovett et al. (1971) and 5 to  $1.2 \times 10^5/g$  obtained by Tabib et al. (1981). On the other hand,

obtained results are lower than that recorded by Abarca et al.,1994 (up to 10<sup>8</sup> CFU/g).

Concerning the differential mould & yeast count in poultry feeds, concentrate mixtures and feed ingredients that recorded in tables (3, 4, 5). The present data showed that the most dominant species are Aspergillus fumigatus, A. flavus, Penicillium chrysogenum, Gibberella fujikuroi, Aspergillus niger and A. terreus. It was observed that A. fumigatus mean count/g was 8.5x10³; 4.8x10³, 9.7x10³; 7.0x10³; 1.0x10⁴; 1.1x10⁴ and 8.4x10³ in laying rations, laying concentrate mixture, broiler concentrate mixture, soybean meal, wheat bran, yellow corn and white corn, respectively. Moreover, the Aspergillus flavus mean count was 8.3x10³;

3.7x103; 8.8x103 in broiler rations, wheat bran and yellow corn, respectively. However, the mean count of Aspergillus niger was 1.4x103; 1.7x103 in the laying concentrate mixture and soybean meal respectively while A. terreus was 7.3x103 in the broiler concentrate mixture. On the other hand, tables 3,4 and 5 revealed that Penicillium chrysogenum mean count/g was  $9.6x10^3$ ;  $7.1x10^3$ ;  $7.1x10^3$ ;  $5.4x10^3$ ;  $8.2x10^3$ ; 1.2x104 and 8.5x103 in laying rations, laying concentrate mixture, broiler concentrate mixture, soybean meal, wheat bran, yellow corn and white corn respectively. Gibberella fujikuroi was isolated from laying rations, broiler rations, white corn by 5.2x103; 1.2x104 and 1.3x104, respectively.

Table (1): Statistical analysis of moisture content in the different poultry feed, concentrates and feed ingredients.

Poultry feeds, concentrates and	No. of ex.		Moisture o	content %
feed ingredients	samples	Min.	Max.	Mean
Poultry rations :-				
Laying rations	9	5.99	6.62	6.33±0.08
Broiler rations(starters)	13	6.12	6.58	6.33±0.06
Concentrates:				
Conc.(Layers)	7	5.64	6.12	5.87±0.06
Conc. (Broilers)	11	4.00	5.88	5.04±0.28
Feed Ingredients :-				
Soyabean meal	13	3.92	8.16	5.61±0.59
yellow corn	14	2.00	7.14	4.58±0.72
White corn	3	6.48	7.84	7.06±0.17
Wheat bran	12	6.12	8.51	7.0±0.29

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Table (2): Statistical mould & yeast count of poultry feeds, concentrates and feed ingredients.

Feeds & feed ingredients	No. of	Tota	l mould & ye	ast count/g
	samples	Min	Max	Mean
Laying rations	9	1.9x10 <sup>4</sup>	9.8x10 <sup>4</sup>	$4.4x10^4 \pm 9.7x10^3$
Broiler rations	13	$4.6 \times 10^3$	1.3x10 <sup>5</sup>	$4.1x10^4 \pm 9.9x10^3$
Conc. layer	7	$5.2x10^3$	5.3x10 <sup>4</sup>	$1.9x10^4 \pm 7.0x10^3$
Conc. broiler	11	$4.4 \times 10^3$	8.7x104	$3.3x10^4 \pm 8.7x10^3$
Soybean meal	13	$6.4 \times 10^3$	4.7x104	$2.2x10^4 \pm 3.8x10^3$
Yellow corn	14	1.6x10 <sup>4</sup>	1.3x10 <sup>5</sup>	$5.2x10^4 \pm 9.7x10^3$
White corn	3	2.6x104	5.3x10 <sup>4</sup>	$4.1 \times 10^4 \pm 9.6 \times 10^3$
Wheat bran	12	1.2x104	8.3x104	$3.7x10^4 \pm 5.4x10^3$

Table (3): Differential mould & yeast count/g in poultry rations.

Fungal species	La	ying rations	(9)	В	roiler rations	(13)
	Min	Max	Mean	Min.	Max.	Mean
Alternaria alternata	0	0	0	0	2x10 <sup>2</sup>	1.5x10
A. chlamydospora	0	0	0	0	2x10 <sup>2</sup>	1.5x10
Aspergillus alutaceus	0	4.0x10 <sup>2</sup>	4.4x10	0	$1.4x10^3$	1.2x10 <sup>2</sup>
A. flavus	8x10 <sup>2</sup>	1.9x10 <sup>4</sup>	4.8x10 <sup>3</sup>	$4.0 \times 10^{2}$	3.2x10 <sup>4</sup>	8.3x10 <sup>3</sup>
A. fumigatus	$1.2 \times 10^3$	2.6x104	8.5x10 <sup>3</sup>	$8.0 \times 10^{2}$	1.6x10 <sup>4</sup>	$6.2 \times 10^3$
A. niger	2.0x10 <sup>2</sup>	2.4x104	4.7x10 <sup>3</sup>	$2.0 \times 10^{2}$	$5.2 \times 10^3$	2.5x10 <sup>3</sup>
A. oryzae	0	0	0	0	$6.0 \times 10^{2}$	4.6x10
A. sydowii	0	$1.2 \times 10^3$	$1.3x10^{2}$	0	0	0
A. tamarii	0	2.0x10 <sup>2</sup>	2.2x10	0	0	0
A. terreus	0	3.8x10 <sup>3</sup>	$9.3x10^{2}$	0	3.4x10 <sup>3</sup>	1.2x10
Cladosporium cladosporioides	0	4.0x10 <sup>2</sup>	6.7x10	0	$4.0x10^{2}$	3.0x10
Emericella nidulans	0	4.0x10 <sup>2</sup>	6.7x10	0	$1.2 \times 10^3$	2.0x10 <sup>2</sup>
E. quadrilineata	0	0	0	0	2.4x10 <sup>3</sup>	1.8x10 <sup>2</sup>
Fennellia flavipes	0	6.0x10 <sup>2</sup>	1.3x10 <sup>2</sup>	0	$2.0 \times 10^{2}$	1.5x10
Gibberella fujikuroi	0	2.1x10 <sup>4</sup>	$5.2x10^3$	0	7.6x10 <sup>4</sup>	1.2x10
Paecilomyces voriotii	0	1.2x10 <sup>4</sup>	$1.8 \times 10^{3}$	0	$6.0 \times 10^{2}$	6.1x10
Penicillium aurantiogriseum	0	$9.0 \times 10^3$	$2.9 \times 10^3$	0	$7.6 \times 10^3$	2.2x10
P. chrysogenum	0	3.1x10 <sup>4</sup>	$9.6 \times 10^{3}$	$2.0x10^{2}$	1.6x10 <sup>4</sup>	7.3x10
P. citrinum	0	8.0x10 <sup>3</sup>	$1.2 \times 10^3$	0	3.8x10 <sup>3</sup>	2.9x10
P. duclauxii	0	2.6x10 <sup>3</sup>	2.9x10 <sup>2</sup>	0	$1.0x10^3$	1.4x10
P. funiculosum	0	2.2x104	$3.9x10^3$	0	$1.2x10^3$	1.4x10
P. variabile	0	0	0	0	$2.0x10^{2}$	1.5x10
Trichoderma harzianum	0	2.0x10 <sup>2</sup>	2.2x10	0	0	0

<sup>\*</sup> N.C.I. = Number of cases of isolations .

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Table (4): Differential mould & yeast count/g in poultry concentrate mixtures.

Fungal species		Conc. layers	(7)	(	Conc. broilers	s (11)
	Min.	Max.	Mean	Min.	Max.	Mean
Acremonium strictum	0	2.0x10 <sup>2</sup>	2.9x10	0	2.0x102	1.8x10
Alternaria alternata	0	0	0	0	$2.4 \times 10^3$	2.2x10 <sup>2</sup>
A. chlamydospora	0	0	0	0	$1.2 \times 10^3$	2.0x10 <sup>2</sup>
Aspergillus alutaceus	0	0	0	Ú	2.0x10 <sup>2</sup>	3.6x10
A. carbonarious	0	2.0x10 <sup>2</sup>	2.9x10	0	0	0
A. flavus	0	2.4x10 <sup>3</sup>	$1.0 \times 10^{3}$	2.0x10 <sup>2</sup>	$4.0x10^3$	1.1x10 <sup>3</sup>
A. fumigatus	8.0x10 <sup>2</sup>	1.1x10 <sup>4</sup>	4.8.x10 <sup>3</sup>	$2.0x10^3$	2.6x104	9.7x10 <sup>3</sup>
A. niger	0	$5.2x10^3$	$1.4x10^3$	0	1.4x10 <sup>4</sup>	2.3x10 <sup>3</sup>
A. oryzae	0	$6.0 \times 10^{2}$	$1.1 \times 10^{2}$	0	$1.0x10^3$	9.1x10
A. sydowii	0	2.4x10 <sup>3</sup>	4.3x10 <sup>2</sup>	0	2.0x10 <sup>2</sup>	1.8x10
A. terreus	0	$8.4 \times 10^3$	$1.6 \times 10^3$	0	6.0x10 <sup>4</sup>	7.3x10 <sup>2</sup>
A. ustus	0	2.0x10 <sup>2</sup>	2.9x10	0	2.0x10 <sup>2</sup>	1.8x10
A. versicolor	0	2.0x10 <sup>2</sup>	2.9x10	0	0	0
Cladosporium cladosporioides	0	$2.0 \times 10^{2}$	8.6x10	0	0	0
Emericella nidulans	0	6.0x10 <sup>2</sup>	$2.0 \times 10^{2}$	0	2.4x10 <sup>3</sup>	4.2x10
E. quadrilineata	0	6.0x10 <sup>2</sup>	$1.1 \times 10^{2}$	0	0	0
Eupenicillium spp.	0	2.0x10 <sup>2</sup>	2.9x10	0	0	0
Eurotium chevalieri	0	2.0x10 <sup>2</sup>	2.9x10	0	0	0
Fennellia flavipes	0	0	0	0	6.2x10 <sup>3</sup>	5.6x10
Gibberella fujikuroi	0	2.0x10 <sup>2</sup>	2.9x10	0	4.0x10 <sup>2</sup>	3.6x10
Humicola minima	0	0	0	0	2.0x10 <sup>2</sup>	1.8x10
Paecilomyces voriotii	0	$1.2 \times 10^3$	$1.7x10^{2}$	0	$1.4x10^3$	1.3x10 <sup>2</sup>
Penicillium aurantiogriseum	$2.0 \times 10^{2}$	$4.2x10^3$	$1.4x10^{3}$	0	$6.8 \times 10^{3}$	1.9x10
P. chrysogenum	$6.0 \times 10^{2}$	2.4x104	$7.1 \times 10^3$	$8.0 \times 10^{2}$	2.2x104	7.1x10
P. citrinum	0	0	0	0	$1.8 \times 10^{3}$	1.6x10
P. duclauxii	0	0	0	0	$3.6 \times 10^3$	3.5x10 <sup>2</sup>
P. funiculosum	0	0	0	0	$7.0 \times 10^3$	8.0x10 <sup>2</sup>
Pleospora tarda	0	$6.0 \times 10^{2}$	8.5x10	0	0	0
Scopulariopsis brevicaulis	0	$2.0x10^{2}$	2.9x10	0	0	0
S. candida	0	4.0x10 <sup>2</sup>	8.6x10	0	0	0

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Fungal species	Soyb	Soybean meal (13)	1 (13)	Wh	Wheat bran (12)	(12)	Yel	Yellow corn (14)	(14)		White corn (3)	(3)
· · · · · · · · · · · · · · · · · · ·	Min	Max	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min	Max	Mean
Acremonium strictum	0	2.0x10 <sup>2</sup>	1.5x10	0	2.4x103	2.0x10 <sup>2</sup>	0	4.0x10 <sup>2</sup>	2.9x10	0	4.0x10 <sup>2</sup>	1.3x10
Alternaria alternata	0	6.0x10 <sup>2</sup>	9.2x10	0	0	0	0	6.0x10 <sup>2</sup>	7.1x10	0		0
A. chlamydospora	0	2.6x103	2.2x10 <sup>2</sup>	0	2.0x10 <sup>2</sup>	1.7x10	0	0	0	0	$1.6 \times 10^3$	5.3x10 <sup>2</sup>
Aspergillus alutaceus	0	0	0	0	2.0x10 <sup>2</sup>	1.7x10	0	1.6x103	2.0x10 <sup>2</sup>	0	0	0
A. clavatus	0	6.0x10 <sup>2</sup>	6.4x10	0	0	0	0	0	0	0		0
). flavus	0	3.4x103	1.0x10 <sup>3</sup>	0	1.2x104	3.7x103	0	3.6x104	8.8x103	8.0x10 <sup>2</sup>	$3.2 \times 10^3$	2.1x10 <sup>3</sup>
. flavus var columnaris	0	0	0	0	6.0x10 <sup>2</sup>	5.0x10	0	0	0	0	0	0
. fumigatus	2.2x103	1.6x104	7.0x10 <sup>3</sup>	3.2x103	2.3x104	1.0x104	3.0x103	2.6x104	1.1x104	1.4x103	1.4x10 <sup>4</sup>	8.4x103
. melleus	0	6.0x10 <sup>2</sup>	4.6x10	0	0	0	0	2.0x10 <sup>2</sup>	2.9x10	0		0
A. niger	0	1.1x104	1.7x103	2.0x102	1.0x104	3.1x10 <sup>3</sup>	0	3.2x104	4.8x103	2.6x103	7.4x10°	4.5x10°
1. oryzae	0	2.0x10 <sup>2</sup>	1.5x10	0	0	0	0	0	0	0	0	0
1. sydowii	0	2.4x103	2.0x102	0	2.0x102	1.7x10	0	1.2x103	8.6x10	0	0	0
1. tamarii	0	$2.0 \times 10^{2}$	1.5x10	0	0	0	0	0	0	0	0	0
1. terreus	0	$3.6 \times 10^3$	6.6x10 <sup>2</sup>	0	5.6x103	$2.3 \times 10^3$	0	2.2x104	2.9x103	0	1.0x10°	3.3x10
A. versicolor	0	$4.0 \times 10^{2}$	3.1x10	0	$1.8 \times 10^{3}$	$2.0 \times 10^{2}$	0	8.0x10 <sup>2</sup>	$1.0 \times 10^{2}$	0	0	0
Chrysosporium tropicum	0	6.0x10 <sup>2</sup>	4.6x10	0	2.0x10 <sup>2</sup>	1.7x10	0	0	0	0	0	0
Cladosporium cladosporioides	0	2.2x103	3.8×102	0	0	0	0	0	0	0	0	0
Emericella nidulans	0	$1.2 \times 10^3$	$2.5 \times 10^{3}$	0	3.2×103	4.8x10 <sup>2</sup>	0	1.2x103	1.4x10 <sup>2</sup>	0	2.6x10°	1.0x10
E. quadrilineata	0	0	0	0	$2.0 \times 10^{2}$	1.7x10	0	0	0	0	0	0
Fennellia flavipes	0	0	0	0	2.4x104	3.2x103	0	0	0	0	0	0
Gibberella fujikuroi	0	7.6x103	1.8x103	0	4.2x103	6.0x10 <sup>2</sup>	0	3.0x104	3.4x103	0	4.1x10 <sup>4</sup>	1.3x104
Humicola grisea	0	2.0x102	1.5x10	0	0	0	0	0	0	0	0	0
Hypomyces chrysospermus	0	6.0x102	6.2x10	0	2.0x10 <sup>2</sup>	1.7x10	0	$9.6 \times 10^3$	6.9x10 <sup>2</sup>	0	2.0x10 <sup>2</sup>	1.3x10°
Mucor racemosus	0	0	0	0	0	0	0	4.0x10 <sup>2</sup>	2.9x10	0	0	0
Paecilomyces voriotii	0	$1.4 \times 10^{3}$	1.7x10 <sup>2</sup>	0	6.0x10	6.7x10	0	5.4x103	$9.4 \times 10^{2}$	0	2.0x10 <sup>2</sup>	6.7x10
Penicillium aurantiogriseum	0	3.8x103	9.4x102	8.0x10 <sup>2</sup>	7.6x10 <sup>3</sup>	3.4x103	0	1.7x104	3.6x103	2.0x102	3.6x103	1.7x103
P. chrysogenum	6.0x10 <sup>2</sup>	2.1x104	5.4x103	$1.4 \times 10^3$	3.6x104	8.2x103	0	5.8x104	1.2x104	3.0x103	1.8x104	8.5x10°
P. citrinum	0	6.0x10 <sup>2</sup>	6.2x10	0	6.0x10 <sup>2</sup>	5.0x10	0	1.0x104	$7.6 \times 10^{2}$	0	0	0
P. duclauxii	0	2.2x103	2.0x10 <sup>2</sup>	0	0	0	0	2.8x103	4.1x10 <sup>2</sup>	0	0	0
P. funiculosum	0	$7.2 \times 10^{3}$	1.1x103	0	4.8x103	6.7x10 <sup>2</sup>	0	3.6x103	7.0x10 <sup>2</sup>	0	0	0
P. oxalicum	0	4.0x102	3.1x10	0	0	0	0	0	0	0	0	0
Pleospora tarda	0	2.0x10 <sup>2</sup>	1.5x10	0	$2.0 \times 10^{2}$	1.7x10	0	0	0	0	0	0
Scopulariopsis brevicaulis	0	6.0x10 <sup>2</sup>	4.6x10	0	0	0	0	0	0	0	0	0
Scytalidium lignicola	0	0	0	0	0	0	0	7.2x10 <sup>3</sup>	5.1x10 <sup>2</sup>	0	0	0
Torula spp.	>			<	-	1 112		>	>		>	>
	•	0	0	0	2.0x10	T. /XIO	0	0	0	0	•	0

Table (5): Differential mould & yeast count/g in poultry feed ingredients.

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Table (6): Frequency distribution and incidence percentages of moulds & yeasts in poultry rations.

Fungal isolates	]	aying rati	ons	Br	oiler ratio	ons	Overall	Overall
		(9 sample	s)	(1	13 sample	s)	incid. %	freq. %
	N.C.I.*	Incid.	Frq.	No. of	Incid.	Frq.		
		%	%	isolates	%	%		
Alternaria alternata	0	0	0	1	1	7.69	0.58	4.54
A. chlamydospora	0	0	0	1	1	7.69	0.58	4.54
Aspergillus alutaceus	1	1.39	11.11	2.	2	15.38	1.74	13.64
A. flavus	9	12.5	100	13	13	100	12.79	100
A. fumigatus	9	12.5	100	13	13	100	12.79	100
A. niger	9	12.5	100	13	13	100	12.79	100
A. oryzae	0	0	0	1	1	7.69	0.58	4.54
A. sydowii	1	1.39	11.11	0	0	0	0.58	4.54
A. tamarii	1	1.39	11.11	0	0	0	0.58	4.54
A. terreus	5	6.94	55.55	10	10	76.92	8.72	68.18
Cladosporium cladosporioides	2	2.78	22.22	1	1	7.69	1.74	13.64
Emericella nidulans	2	2.78	22.22	3	3	23.08	2.91	22.73
E. quadrilineata	0	0	0	1	1	7.69	0.58	4.54
Fennellia flavipes	2	2.78	22.22	1	1	7.69	1.74	13.64
Gibberella fujikuroi	6	8.33	66.66	9	9	69.23	8.72	68.18
Paecilomyces voriotii	3	4.17	33.33	2	2	15.38	2.91	22.73
Penicillium aurantiogriseum	7	9.72	77.78	10	10	76.92	9.88	77.27
P. chrysogenum	8	11.11	88.88	13	13	100	12.21	95.45
P. citrinum	2	2.78	22.22	1	1	7.69	1.74	13.64
P. duclauxii	1	1.39	11.11	2	2	15.38	1.74	13.64
P. funiculosum	3	4.17	33.33	2	2	15.38	2.91	22.73
P. variable	0	0	0	1	1	7.69	0.58	4.54
Trichoderma harzianum	1	1.39	11.11	0	0	0	0.58	4.54
Total	72			100				

<sup>\*</sup> N.C.I. = Number of Cases of Isolations .

 $Table (7): Frequency\ distribution\ and\ incidence\ percentages\ of\ moulds\ \&\ yeasts\ in\ poultry\ concentrate\ mixtures.$ 

Fungal isolates		concent			iler concen		Overall incid. %	Overall freq. %
	N.C.I.*		Frq.	No. of isolates	Incid.	Frq.	_	
Acremonium strictum	1	1.67	14.28	1	1.19	9.09	1.39	11.11
Alternaria alternata	0	0	0	1	1.19	9.09	0.69	5.55
A. chlamydospora	0	0	0	3	3.57	27.27	2.08	16.67
Aspergillus alutaceus	0	0	0	2	2.38	18.18	1.39	11.11
A. carbonarious	1	1.67	14.28	0	0	0	0.69	5.55
A. flavus	4	6.67	57.14	11	13.09	100	10.42	83.33
A. fumigatus	7	11.67	100	11	13.09	100	12.50	100
A. niger	6	10	85.71	9	10.71	81.81	10.42	83.33
A. oryzae	2	3.33	28.57	1	1.19	9.09	2.08	16.67
A. sydowii	2	3.33	28.57	1	1.19	9.09	2.08	16.67
A. terreus	5	8.33	71.43	9	10.71	81.81	9.72	50.0
A. ustus	1	1.67	14.28	1	1.19	9.09	1.39	11.11
A. versicolor	1	1.67	14.28	0	0	0	0.69	5.55
Cladosporium cladosporioides	3	5.0	42.86	0	0	0	2.08	16.67
Emericella nidulans	3	5.0	42.86	0	0	0	2.08	16.67
E. quadrilineata	2	3.33	28.57	4	4.76	36.63	4.17	33.33
Eupenicillium spp.	1	1.67	14.28	0	0	0	0.69	5.55
Eurotium chevalieri	1	1.67	14.28	0	0	0	0.69	5.55
Fennellia flavipes	0	0	0	1	1.19	9.09	0.69	5.55
Gibberella fujikuroi	1	1.67	14.28	1	1.19	9.09	1.39	11.11
Humicola minima	0	0	0	1	1.19	9.09	0.69	5.55
Paecilomyces vorietii	1	1.67	14.28	1	1.19	9.09	1.39	11.11
Penicillium aurantiogriseum	7	11.67	100	9	10.71	81.81	11.11	88.89
P. chrysogenum	7	11.67	100	11	13.09	100	12.50	100
P. citrinum	0	0	0	1	1.19	9.09	0.69	5.55
P. duclauxii	0	0	0	2	2.38		1.39	11.11
P. funiculosum	0	0	0	3	3.57	18.18	2.08	16.67
Pleospora tarda	1	1.67	14.28	0	0	27.27	0.69	5.55
Scopulariopsis brevicaulis	2	3.33	28.57	0	0	0	1.39	11.11
S. candida	1	1.67	14.28	0	0	0	0.69	5.55
Total	60			84		0		

<sup>\*</sup> N.C.I. = Number of Cases of Isolations .

Table (8): Frequency distribution and incidence percentages of isolated mould & yeast from poultry feed ingredients.

Fungal Isolates	Soyl	Soybean meal (13)	ıl (13)	M	Wheat bran (12)	n (12)	Yel	Yellow corn (14)	(14)	×	White corn (3)	(3)	Overall	Overall
	N.C.I	Inc. %	Frq. %	NCI	Inc%	Frq. %	N.CI	Inc%	Frq.%	N.CI	Inc %	Frq. %	Incid. %	Frq. %
Acremonium strictum	1	0.88	69.4	-	1	8.33	1	6.0	7.14	1	4.17	33.33	1.15	9.52
Alternaria alternata	3	2.65	23.1	0	0	0	7	1.8	14.28	0	0	0	1.44	11.9
A. chlamydospora	7	1.77	15.38	1	1	8.33	0	0	0	1	4.17	33.33	1.15	9.52
Aspergillus alutaceus	0	0	0	1	1	8.33	7	1.8	14.28	0	0	0	98.0	7.14
A. clavatus	1	0.88	7.69	0	0	0	0	0	0	0	0	0	0.29	2.38
A. flavus	10	8.8	76.9	11	111	79.16	12	10.81	85.71	3	12.5	100	10.34	85.71
A. fiavus var columnaris	0	0	0	1	1	8.33	0	0	0	0	0	0	0.29	2.38
A. fumigatus	13	11.5	100	12	12	100	14	12.61	100	3	12.5	100	12.1	100
A. melleus	1	0.88	7.69	0	0	0	7	1.8	14.28	0	0	0	98.0	7.14
A. niger	30	7.1	61.54	12	12	100	12	10.81	85.71	3	12.5	100	10.1	83.33
A. oryzae	1	0.88	7.69	0	0	0	0	0	0	0	0	0	0.29	2.38
A. sydowii	2	1.77	15.38	1	1	8.33	1	6.0	7.14	0	0	0	1.15	9.52
A. tamarii	1	0.88	7.69	0	0	0	0	0	0	0	0	0	0.29	2.38
A. terreus	7	6.19	53.8	11	11	19.16	17	10.81	85.71	1	4.17	33.33	8.91	73.81
A. versicolor	1	0.88	7.69	17	7	16.67	7	1.8	14.28	0	0	0	1.44	11.9
Chrysosporium tropicum	1	0.88	7.69	1	-	8.33	0	0	0	0	0	0	0.57	4.76
Cladosporium cladosporioides	7	6.19	53.8	0	0	0	0	0	0	0	0	0	2.01	16.67
Emericella nidulans	w	4.42	38.5	4	4	33,33	8	2.7	21.43	7	8.33	1.99	4.02	33.33
E. quadrilineata	0	0	0	1	1	8.33	0	0	0	0	0	0	0.29	2.38
Fennellia flavipes	0	0	0	4	4	33,33	0	0	0	0	0	0	1.15	9.52
Gibberella fujikuroi	3	2.85	23.1	4	4	33.33	9	5.4	42.86	1	4.17	33.33	4.02	33,33
Humicola grisea	1	0.88	7.69	0	0	0	0	0	0	0	0	0	0.29	2.38
Hypomyces chrysospermus	2	1.77	15.38	1	1	8.33	1	6.0	7.14	7	8.33	1.99	1.72	14.28
Mucor racemosus	0	0	0	0	0	0	1	6.0	7.14	0	0	0	0.29	2.38
Paecilomyces voriotii	4	3.54	30.77	2	7	16.67	4	3.6	28.57	1	4.17	33.33	3.16	29.19
Penicillium aurantiogriseum	11	9.73	84.6	12	12	100	12	10.81	85.71	3	12.5	100	10.92	90.48
P. chrysogenum	13	11.5	100	12	12	100	13	11.71	92.86	3	12.5	100	11.78	97.62
P. citrinum	2	1.77	15.38	1	1	8.33	7	1.8	14.28	0	0	0	1.44	11.9
P. duclauxii	7	1.77	15.38	0	0	0	3	2.7	21.43	0	0	0	1.44	11.9
P. funiculosum	7	61.9	53.8	3	3	25.0	w	4.5	35.71	0	0	0	4.31	35.71
P. oxilacum	1	0.88	7.69	0	0	0	0	0	0	0	0	0	0.29	2.38
Pleospora tarda	1	0.88	7.69	1	1	8.33	0	0	0	0	0	0	0.57	4.76
Scopulariopsis brevic aulis	1	0.88	4.69	0	0	0	0	0	0	0	0	0	0.29	2.38
Scytalidium lignicola	0	0	0	0	0	0	I	6.0	7.14	0	0	0	0.29	2.38
Torula sp.	0	0	0	-	1	8.33	0	0	0	0	0	0	0.29	2.38
Yeasts	1	0.88	7.69	0	0	0	0	0	0	0	0	0	0.29	2.38
Total	113			100			1111			24				

The isolated mycoflora from mixed poultry feeds pose problems due to the ability of some of them to invade the animal tissues (A. fumigatus) as well as their ability to grow on feed, causing their spoilage beside production of mycotoxins (Moreno-Rome & Fernandez, 1986). So it is necessary to note the high prevalence of these mycoflora in general and with special emphasis on which comprise the most important and well known mycotoxin producing fungi affecting the mixed poultry feeds and their ingredients.

The frequency distribution and incidence percentages of the isolated fungi were shown in tables (6-8). A total of 20 genera; 42 species and one species variety were isolated from all examined samples. The most prevelant genera were Aspergillus that represented by overall frequency percentages of 100% in both of broiler rations, followed by laving and Cladosporium Penicillium spp., Gibberella spp., Paciliomyces spp., Emericella Hypomyces spp., Fennellia spp., Alternaria spp., Trichoderma spp. and Mucor species. The remaining identified genera had lower frequencies of isolation and they were designated as mycoflora of rare occurrence in poultry mixed feeds and their ingredients (Ogundero, 1980).

A. fumigatus was the dominant species isolated from all examined samples with an overall frequency of 100 %. The fungus causes respiratory disease in man and poultry (Lacey, 1975). The ingestion of spoiled poultry feeds with the toxic fungal metabolites is responsible for the poultry toxicosis (Lovett,

1972). Furthermore, A. fumigatus is very dangerous for patients with reduced immunological competence as a consequence of chronic diseases including diseases of lung, liver and kidney, leukemia, tuberculosis, diabetes and kidney and AIDS (Reiss, 1995).

A. flavus is considered as a dominant species in the poultry feeds, concentrate mixtures and feed ingredients (tables 3-5). The mean values/g of A. flavus were 8.8x103,  $8.3 \times 10^3, 4.8 \times 10^3, 3.7 \times 10^3, 2.1 \times 10^3, 1.1 \times 10^3,$ 1.0x10<sup>3</sup> and 1.0x10<sup>3</sup> in yellow corn; broiler rations; laying rations; wheat bran; white corn; broiler concentrate mixtures; layer concentrate mixture and soybean meal respectively. It was recovered with an over all frequency of 100% from laying and broiler rations; 85.71% from poultry feed ingredients 83.33% from laying and broiler concentrate mixtures. A. flavus is considered the main fungus that produces aflatoxins mycotoxicosis which has been causing recognized throughout the world as a major problem in poultry and animal industry ( Mirocha & Christensen, 1974 and Abarca et al., 1994).

Generally Aspergillus species are incriminaed in poultry mycotoxicosis as many of them are toxic producer including A. flavus, A. fumigatus, A. terreus, A. clavatus and A. glaucus (Forgacs, 1966 and Lovett, 1972). Furthermore, avian aspergillosis which is caused by several species of Aspergilli as A. fumigatus, A. nidulans and A. niger (Singh & Singh, 1970; Hubbert et al., 1975 and Muller, 1984).

Penicillium species were recovered with high frequency distributions as P. chrysogenum which showed an overall frequency of 100; 97.62 and 95.45% in poultry concentrate mixtures, feed ingredients and poultry rations respectively. Penicillum spp. are implicated in pulmonary mycosis, gastrointestinal disturbances and poultry mycotoxicosis (Christensen et al., 1968 and Lovett, 1972).

The two species of Alternaria that could be isolated were Alternaria alternata and Alternaria chlamydospora. A. alternata was recovered with overall frequency of 11.9; 5.55 and 4.54% from feed ingredients, concentrate mixtures and poultry rations respectively. On the other hand, A. chlamydospora was recorded with overall frequencies of 16.67; 9.52 and 4.54% in concentrate mixtures, feed ingredients and poultry rations respectively. Alternaria spp. are reported as saprophytes however, they have been recorded as opportunistic causative agent of poultry mycotoxicosis (Abrams, 1965 and Forgacs et al., 1962).

The highest overall frequency of Gibberella fujikuroi was observed in poultry rations (68.18%), followed by feed ingredients (33.33%). The fungus produces estrogenic substance which inhibits ovulations and significantly reduce egg production and feed consumption in laying hens (Schaible, 1970 and Adams & Tuite, 1976).

The remaining identified genera including Cladosporium cladosporioides; Mucor racemosus;

Paeciliomyces voriotii; Scopulariopsis brevicaulis and Trichoderma harzianum occurred with variable overall frequencies in the examined samples. From the hygienic point of view Cladosporium spp. are implicated in some cases of dermatitis in chickens and poultry mycotoxicosis (Christensen et al., 1968 and Lovett, 1972). The Mucor spp. representing health hazard of the birds as they incriminated in mucormycosis of birds and man (Jand & Dhillon, 1973 and Al-Doory, 1980). Paeciliomyces spp. are incriminated in some pulmonary affections of chickens and usually isolated from the lungs and air sacs of diseased birds (Chute et al., 1956 and Eckman & Morgan, Furthermore, Paeciliomyces were implicated in poultry mycotoxicosis (Abrams, 1965 and Forgacs, 1966). Scopulariopsis spp. are incriminated in pulmonary affection of chickens and in some cases of mycotoxicosis (Chute, et al., 1956; Christensen et al., 1968 and Lovett, 1972). Moreover, Scopulariopsis have been reported as a causative agent for nail affections and in deep seated granulomatus lesions (Al-Doory, 1980). Trichoderma spp. were recorded as a pathogen to young broiler chicks and usually isolated from the lungs and air sacs beside they are incriminated in avian mycotoxicosis (Chute, et al., 1956; Christensen et al., 1968 and Lovett, 1972). Finally the fungal contamination of poultry feeds and their ingredients leads to losses of their nutrient constituents beside the determintal effects on poultry health and production, so, it is of urgent need to evaluate and control the mycological quality (Buhatel et al., 1982).

#### **CONCLUSION:**

The results of this study showed a high incidence of a wide variety of fungi from the poultry feeds and their ingredients. Presence of Aspergillus flavus and others in some of these feeds may include pathogenic strains producing aflatoxins which may constitutes a potential health hazards to poultry and man. Additional comprehensive monitoring programmes with regular intervals must be considered to evaluate the hygienic fitness of such poultry feeds and their ingredients to overcome and minimize the economic losses in the Egyptian poultry farms and to safeguard health. Moreover, the authors recommended the prevention of initial mould growth by using good hygienic stores for the poultry feeds and the use of proper antifungal agents. In this respect the use of mould inhibitors in the mixed feeds may be of economical importance during periods when feeds may have to be stored under conditions where heating is the major significant factor (Hafez et al.,1995).

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# التقييـــــــم الفطــــرى لعلائق الدواجــــن وبعض المركزات والمكونات خاصتما

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أجريت هذه الدراسة للوقوف على الحالة الصحية لبعض علائق الدواجن ومكوناتها وذلك بالتقييم الفطرى لها . وإستيفاءاً لهذا الغرض فإنه قد تم تجميع وإجراء الفحوص الميكولوجية لإجمالي عدد ٨٢ عينة شملت ٢٢ عينة عليقة منتج نهائي ، ١٨ عينة مركزات أعلاف هذا بالإضافة إلى ٤٢ عينة من المكونات الأولية لهذه العلائق والتي تم تجميعها من وحدات متخصصة لإنتاج علائق الدواجن المختلفة .

وقد أظهرت النتائج وجود محتوى مائى تمثل فى متوسطات قيم الرطوبة النسبية لبعض هذة العلائق ومكوناتها والتى تراوحت بين  $4.0.1 \pm 1.0.1 \pm 1.0.1 \pm 1.0.1 \pm 1.0.1 \pm 1.0.1 \pm 1.0.1 \pm 1.0 \pm 1$