ORIGINAL ARTICLE **Mirror of Research in Veterinary Sciences and Animals MRVSA/ Open Access DOAJ**

Determination of penicillic acid in Poultry food in Diyala Governorate

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ARTICLE INFO
Received: 05.10.2016
Revised: 15.10.2016
Accepted: 30.10.2016
Publish online: 25.11.2016

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Abstract

Contamination of the poultry feed with fungi and its toxin products can lead to nutrient losses and detrimental effects on animal health and production. This study was designed to isolate the fungi especially *Penicillium spp*. and to determine the concentrations of Penicillic acid (PA) levels in poultry food samples collected from different Layer and Broiler chicken farms in Diyala governorate. Food samples were collected from four sectors farms (Alkhalis, Baquba, Baldrooz and Almuqdadia) from Diyala governorate in the period extended from November 2015 to March 2016. High-performance liquid chromatography (HPLC) was used to analysis food samples. *Mucar spp., Rhizopus spp., Absidia spp., Aspergillus niger, Penicillium spp., Aspergillus ochraceus, Aspergillus fumigatus, Chrysonilia sitophila, Aspergillus flavus, Fusarium spp., Apophysmyces elegans, Paecilomyces lilacinus, Scopulariopsis, Circinella* were isolated from different food samples. Moreover, *Candida spp., Rhodotorula rubra, Geotricum candid* and *Cryptococcus neoformans* were the most isolated yeast species. All feed samples were positive for PA. The highest levels of penicillic acid in Alkhalis, Baquba, Baldrooz and Almuqdadia were (53.47±2.75), (49.62±2.43), (48.39±2.35) and (47.29±2.37) respectively in Layer chicken and (53.66±2.79), (49.99±2.62), (48.25±2.35) and (47.23±2.42) respectively in broilers. No significant differences ($P \leq 0.05$) was seen in the levels of PA among the food samples from the four different sectors. In conclusion, this study approved the presence of mycotoxigenic fungi in the poultry foods that increases the risk of mycotoxin poisons for the animal health and its implications for human health. The authors recommend another future study in another farms in Diyala including a large number of samples to understand the actual situation of the fungi toxin products.

To cite this article: Noor Th. Talib; Dalia A. Abdul-Shaheed. (2016). Determination of penicillic acid in Poultry food in Diyala Governorate MRVSA. 5 (3), 24-30.
DOI: 10.22428/mrvsa. 2307-8073.2016. 00534.x

Keywords: Poultry feed, Penicillic acid, *Penicillium spp*. toxin.

Introduction

The secondary metabolites of filamentous fungi are the Mycotoxins that occur in the food commodities. It plays an important source of contamination for different types of food crops throughout the food chain (Reddy *et al.*, 2010). Mycotoxins have several

implications. The great economic losses are one of the important implications, moreover, threaten to the human and animal health. Mycotoxins are a cause of a wide variety of harmful effects when they ingest. They are known to cause: general toxic effects, immune system suppression, mutations, cancer, and teratogenic affects (mutations in offspring) (Mishra and Sopori, 2012). Penicillic acid is a mycotoxin that produced by many *Penicillium* and *Aspergillus* species, especially *Penicillium puberulum* (Alsberg and Black, 1913). *P. aurantiogriseum*, *P. carneum*, *P. cyclopium*, *P. freii*, *P. melanoconidium*, *P. neoechinulatum*, *P. polonicum*, *P. radicicola*, *P. tulipae* and *P. viridicatum* are also producing of PA particularly by the members of the *Aspergillus ochraceus*. PA has been reported to express a variety of biological activities, including hepatotoxicity, kidney damage, apoptosis and cause malignant, transplantable tumors in rats and mice (Frisvad *et al.*, 2004). It was found to interfere with protein formation due to its effect on the metabolism of carbohydrate enzymes, nucleic acids and increased glycogen level that observed during toxicosis. It is also affected on the lipid metabolism leading to lowered levels of total lipids (Pandiyan *et al.*, 1987) and changed the expression of Bovine Macrophages enzymes that involved in epigenetic regulation (Se-Young *et al.*, 2013). Review of literature regarding mycotoxin in Iraq / Diyala governorate revealed scarce information. Therefore, this study intends to isolate the fungi especially *Penicillium spp*. and to determine the concentrations of PA levels in poultry food samples from different Layer and Broiler chicken farms in Diyala governorate.

Material and Methods

This study is a collaboration research between the College of Veterinary Medicine / University of Baghdad and the Ministry of Science and Technology.

Collection of samples

Total, 80 poultry food samples collected from 4 sectors farms in Diyala governorate (Alkhalis, Baquba, Baldrooz and Almuqdadia) in the period extended from November 2015 to March 2016. The food samples included 40 samples for each Layer and Broiler chicken (10 samples for each sector and two samples for each month). About 1 kg of each food sample was collected and ground in a mill and stored in paper bags at room temperature until cultured on mycological media and analyze by HPLC.

Media used for Isolation and Identification of Fungi

Potato Dextrose Agar powder (Accumix) company prepared by suspension of 39 gm in a liter of distilled water. Later on, the suspension sterilized by autoclaving at 121˚C under 15 pounds/inch 2 for 15 min. When the medium cooled down enough, Chloramphenicol (250mg/L) was added to the medium and swirl to mix. Then, the medium poured into Petri plates. Macro- and microscopic criteria were used to identify the genus of fungi according to Leslie and Summerell, (2006).

Determination of Penicillic Acid levels in the food samples

All reagents and solvents prepared for HPLC for analytical determination and standard PA from Santa Crusz Company (USA) were used to standardize the machine. The analysis was performed by an Agilent 1200 Series HPLC with a diode array detector (DAD). The analytical column was an Agilent ZORBAX Eclipse Plus C18/ 2.1 mm×100 mm, 1.8μm. Nylon Syringe Filter (Agilent 0.22 μm) was used to filter the sample solution before HPLC.

Preparation of standard Penicillic Acid

Standard PA was prepared by dissolving 1 mg of PA in 1 ml of methanol and kept at -20 °C.

Sample extraction

The procedures was done according to the method described by Gong *et al.,* (2012). Ten grams of each sample ground in a mill and placed into a 100 ml centrifuge tube with 40 ml of (84:16, v/v) acetonitrile : water. Later on, the sample was homogenized for 3 min with high-speed homogenizer and was centrifuged at 10,000 R/min/10 minutes under 4ºC. The supernatant solution was evaporated to dryness under a stream of nitrogen at 50ºC. The residue was re-dissolved in 8 ml of mixture of ethylacetate/cyclohexane (50:50, v/v) and passed through a 0.45 μm nylon filter and then 20 μl were subjected to HPLC analysis.

Statistical Analysis

Mean data values presented with their standard error (mean ±SE) and analyzed using one-way analysis of variance (ANOVA) followed by multiple mean comparisons (SPSS. (2008).

Results and Discussion

Different fungi were isolated in different isolation rate (percentages) from poultry food (Table. 1). The isolation rates of *Mucar spp., Rhizopus spp., Absidia spp.* and *Aspergillus niger* were (10.6%), *Penicillium spp.* (9.6%), *Aspergillus ochraceus* (6.4%), *Aspergillus fumigatus* and *Chrysonilia sitophila* (4.3%), *Aspergillus flavus* and *Fusarium spp.* (3.2%). Moreover, *Apophysmyces elegans, Paecilomyces lilacinus, Scopulariopsis* percentage was (2.1%) and (1.0%) for *Circinella*. On the other hand, the percentages of yeasts were 6.4%, 5.3%, 4.3% and 3.2% for *Candida spp., Rhodotorula rubra, Geotricum Candida* and *Cryptococcus neophormans* respectively. The results of the current study confirmed that the poultry food infected with fungi.

The incidence of fungi occurs due to the inconvenient environmental storage condition that enhances the growth of fungi. These findings are in agreement with the previous study (Magan and Aldred, 2007), who reported that climate represents the key agro-ecosystem driving force of fungal colonization and mycotoxin production. Moisture and temperature are the most important parameters for fungal growth and mycotoxin production. Storage temperatures between 25°C and 30°C and a relative humidity of 97% favor the growth of fungi and production of toxins during storage. Moreover, the previous

study approved that about 25% of the world storages of cereals were affected by molds every year (Freitas-Silva *et al.*, 2011). Physical damage also occurs due to insect activities on grains (Al- Ani, 2008). The development of microorganisms leads to the growth of molds due to the changes that take places with the complex enzymatic requirement. All these changes permit the taking of necessary elements from the substratum for cellular development and the energy for their vital processes in restrictive conditions of low humidity, temperature, pH, in the absence of growth factors (Pitt, 2011).

(Table 1) The percentage of fungal contamination of poultry feed for Layer and Broiler chicken

Fungus spp.	No. of Isolates	percentage%
Moulds		
Mucar spp.	20	10.6
Rhizopus spp.	20	10.6
Absidia spp.	20	10.6
Aspergillus niger	20	10.6
Pencillium spp.	18	9.6
Aspergillus ochraceus	12	6.4
Aspergillus fumigatus	8	4.3
Chrysonilia sitophila	8	4.3
Aspergillus flavus	6	3.2
Fusarium spp.	6	3.2
Apophysmyces elegans	4	2.1
Paecilomyces lilacinus	4	2.1
Scopulariopsis	4	2.1
Circinella	2	1.0
Yeasts		
Candida spp.	12	6.4
Rhodotorula rubra	10	5.3
Geotricum candidum	8	4.3
Cryptococcus neophormans	6	3.2
Total	188	100%

The HPLC analyses showed that all food samples were positive for penicillic acid (Table. 2). The highest levels of penicillic acid in Alkhalis, Baquba, Baldrooz and Almuqdadia were (53.47±2.75), (49.62±2.43), (48.39±2.35) and (47.29±2.37) respectively in Layer chicken and (53.66±2.79), (49.99±2.62), (48.25±2.35) and (47.23±2.42) respectively in broilers (Figure.1). Meanwhile, no significant differences ($P \leq 0.05$) observed in the levels of penicillic acid among the samples of the four different sectors. The presence of different microflora in animal food considers as an indicator of the increased activity of microorganisms and production of various metabolism products. The most harmful products are mycotoxins. In the poultry production, food is the key vector for introducing of mycotoxins into flocks of birds (Vesna *et al.*, 2014 ;). Zaki *et al.*, (2012) suggested that the temperature, water activity, and oxygen are the most important factors for growth of fungi and mycotoxin production besides the presence of nutrients. Often, fungi invade only the minor fraction of feed particles with the appropriate condition for growth such as enough water content and aeration. Fungal growth is also affected by the substrates

characteristic features such as the physical including Water activity, oxygen availability and surface area; and chemical like carbohydrates, fat, protein, trace elements and amino acid composition (Russell and Nelson. 2010). The previous study approved that some substrates were susceptible to colonization. Moreover, the environmental conditions including temperature, water activity, pH and atmospheric air (oxygen) can increase the vulnerability of the fungi to the substrate (Moss, 1991). Northolt, (1979) also explained the combined effects of water activity and temperature on growth of fungi and the production of penicillic acid by *Penicillium cyclopium* and *Penicillium martensii* and they found that the minimum water activity for PA production was 0.97, while the temperature range (4-31) ^{o}C.

Table. 2. Shows the concentration levels of penicillic acid (ppb) in Poultry Feed samples of Laying and Broiler chickens in sectors of Diyala province by HPLC

Sector	Laying chickens			Broiler chickens		
	No. of sample	Range	Mean ± SE	No. of sample	Range	Mean± SE
Alkhalis	10	41.99-63.04	53.47±2.75	10	42.13-63.17	53.66±2.79
Baquba	10	40.36-60.01	49.62±2.43	10	40.01-61.23	49.99±2.62
Baldrooz	10	39.82-58.74	48.39±2.35	10	39.77-58.91	48.25±2.35
Imuqdadia	10	38.79-57.91	47.29±2.37	10	38.81-57.99	47.23±2.42

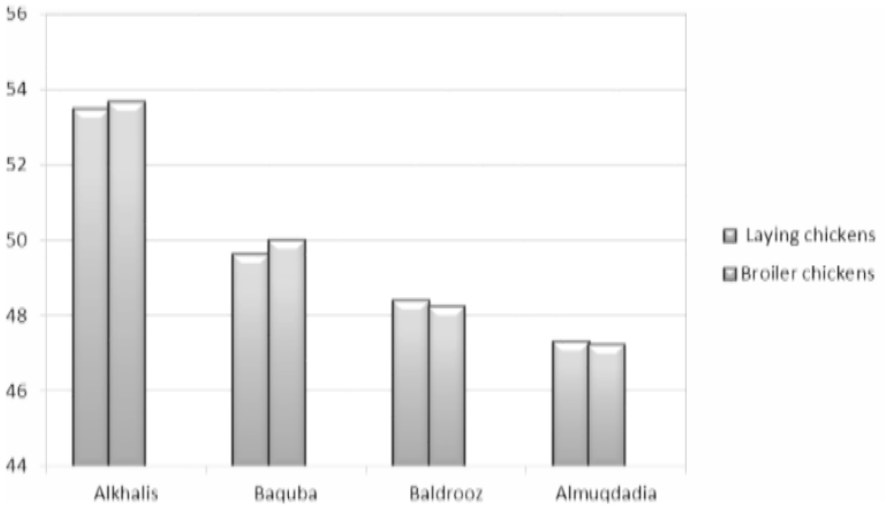

Figure. 1. Shows the mean concentration levels of penicillic acid in Poultry Feed samples of Laying and Broiler chickens in sectors of Diyala province.

In conclusion, the results of this study approved the presence of mycotoxigenic fungi in the poultry foods that increases the risk of mycotoxin and its implications on the human and animal health. The authors recommend more future study in another farms in Diyala including a large number of samples to investigate the factors that enhance the growth of these fungi to produce the mycotoxin.

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