Mycoflora and naturally occurring mycotoxins in poultry feeds in Argentina

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Abstract

The purpose of this work was to determine the mycoflora and mycotoxins natural incidence in poultry feeds from 2 factories in Río Cuarto, Córdoba. One hundred and thirty samples were taken from May/1996 to May/1997. The most dominant species isolated of poultry feed samples belonged to the genera Aspergillus spp 85% and Fusarium spp 70%. From Aspergillus genus eleven species were identified and A. flavus was the most frequent. Nine species were identified from the Fusarium genus and the predominant was F. moniliforme. Penicillium ranked third in the number of isolated cases. From this genus twelve species were collected of which P. brevicompactum (15%), P. restrictum (14%) and P. purpurogenum (12%) were the most common.

The most significant mycotoxin from poultry feeds was aflatoxin B₁ (AFB₁) found in 48% of the samples, with levels ranging from 10 to 123 ng/g. For zearalenone (ZEA) the levels were 327 to 5,850 ng/g and DON was not detected from the samples. Due to the fact that in Argentina there is little information about this topic, these data on poultry feeds in our region would be of worldwide interest.

Key words: poultry feeds, Fusarium, Aspergillus, Penicillium, mycotoxins

Introduction

The presence of mould and mycotoxins in poultry feeds result from the raw material used in their production. Mould and mycotoxins contamination of the raw materials occur during the pre-harvest (field-produced fungi) and/or the post-harvest (storage-produced fungi) periods. During these periods, temperature and humidity play an important role in the growth of fungi and mycotoxins contamination [1].

The intake of very low levels of mycotoxins causes overt mycotoxicosis but also leads to the impairment of immune and acquired resistance to infections causing health problems which lead to economic losses in the form of decreased productivity [2].

In Argentina there is no information available about the natural occurrence of mycotoxins and mycoflora contamination of feedstuffs. It was demonstrated that preliminary data on this topic in our region (south of Córdoba, Argentina) have brought about important changes in quality of chicken feeds over time. The results suggest some kind of relationship between some of mycotoxins producer genus frequencies and the occurrence of their related mycotoxin [3]. These species present different metabolic profiles and characteristic toxicogenics. Therefore, their correct identification is fundamental to orientate the search for possible mycotoxins. Besides, the study of the incidence of toxicogenic species and their mycotoxins is important to guarantee the quality not only of hygiene but also nutritional conditions of poultry feeds.

To get representative results of the problem, the studies of incidence should take place for 3 years or more, therefore the aim of this work was to continue with the study of the incidence of toxicogenic fungi and their mycotoxins in poultry feeds during a period of thirteen months starting in May 1996.
Materials and methods

Sampling

The samples of poultry feeds were collected from 2 factories (chosen at random) out of 5 checked in a previous study [3]. They are located in the department of Río Cuarto from Córdoba province, Argentina. A total of 130 samples (5 samples per month from each factory) were collected from May to May 1996–1997. The samples of 10 kg each, were taken from each factory during the production process. These primary samples were homogenized and quartered to obtain a 1 kg laboratory sample. Moisture content was measured immediately and the samples were stored at 4 °C for fungal and mycotoxin analysis, this was done the day after collection.

Moisture content of poultry feeds

Two grams of sample were dried in an oven with forced air circulation for 16 hours at 80 °C. The samples (five replicates) were weighed and the initial water content was determined.

Mycoflora determination

Quantitative enumeration of fungal propagules was done on solid media using the surface-spread method by blending 10 g portions of each sample with 90 ml of 0.1% peptone water solution. Serial dilutions of $10^{-2}$–$10^{-4}$ concentration were made from each material and 0.1 ml aliquots were inoculated by triplicates on different media. Dichloran rose bengal chloramphenicol agar (DRBC) was used for general fungal enumeration [4]. Aspergillus flavus and A. parasiticus agar medium (AFPA) was used for the detection of potentially aflatoxigenic fungi [5] and Nash–Snyder medium for isolation of Fusarium species [6].

Plates contained media DRBC and AFPA were incubated at 28 °C for 7 days. The Nash–Snyder plates were incubated at 24 °C for 7 days under 12/12 hours photoperiod cold white and black fluorescent lamps. On the last day of incubation, plates that only contained 10–100 CFU were used for counting and the results were expressed as CFU per gram of sample. Fungal colonies were selected for identification according to the methods proposed for each genus [5, 6, 9].

Fungal colonies identified like Aspergillus and Penicillium were subcultured in potato dextrose agar (PDA) and Fusarium in carnation leaf agar (CLA) for posterior identification in species.

Taxonomic identification of all colonies considered as different was achieved through macroscopic and microscopic studies followed by standard tests which were related to the genera of each particular group of fungi. Aspergillus species were identified according to taxonomic schemes proposed by Pitt and Hocking, Raper and Fennell [5, 6]. Penicillium and Fusarium species according to Pitt et al. [10] and Nelson et al. [7] respectively.

Mycotoxin analysis

Aflatoxins, zearalenone and deoxynivalenol analyses were performed by TLC, following the methodology proposed by Richard et al. [10] for feedstuffs.

Statistical analysis

The non-parametric test of Mam–Whitney was used to compare the means of four variables: count mold, moisture content, AFB1 and ZEA between two periods, (Period 1 May–September 1996, Period 2 October 1996–April 1997). The means of two periods were treated as independent samples because the taking for granted of normality achievement was not verified [12]. Data analysis for each sample ($n = 10$ per month) was done in triplicate.

Results

The count of isolated genera fungi was defined as the percentage of samples in which each fungus was present. Mycological survey of 130 samples of poultry feed showed the presence of 18 genera of filamentous fungi (Figure 1). The species belonging to Aspergillus spp (85%) and Fusarium spp (70%) genera were the most frequent. These genera were represented by a great variety of species and many of them were identified.

Aspergillus was the most common genus and eleven species were identified. Among Aspergillus spp, A. flavus was the most frequent (36%) (Figure 2).

Fusarium was the second most frequent genus and nine species were identified. The predominant species was F. moniliforme (51%) (Figure 3).