

The mycoflora and toxicity of feedstuffs from a production plant in Córdoba, Argentina.

Magnoli C¹, Chiacchiera SM², Miazzi R³, Palacio G⁴, Angeletti A¹, Hallak C¹ and Dalcero A¹

¹ Dpto. de Microbiología e Inmunología,

² Dpto. de Química y Física,

⁴ Dpto. de Matemáticas. Facultad de Ciencias, Exactas Físico-Químicas y Naturales.

³ Dpto. de Producción Animal. Facultad de Agronomía y Veterinaria. Universidad Nacional de Río Cuarto.

* Address for correspondence: Dra. Ana M. Dalcero. Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas Físico Químicas y Naturales, Universidad Nacional de Río Cuarto. Ruta Nacional N° 36 Km 601 (5800) Río Cuarto, Córdoba, Argentina.

Phone 54-358-4676113; Fax: 54-358-4676231. E-mail: adalcero@exa.unrc.edu.ar

Abstract

Feedstuffs used for poultry nutrition in Argentina were analyzed for fungal flora and natural incidence of mycotoxins. Survey of 120 samples of poultry feeds, taken from May 1998 to April 1999, showed the presence of 15 genera of filamentous fungi. The predominant genera were *Fusarium* spp. and *Penicillium* ssp., isolated in 67.5 % of the samples, followed by *Aspergillus* spp. (57.5 %). Yeast, were significantly isolated from most of the samples. Species identification was carried down for the toxigenic genera. Fungal total counts of poultry feeds ranged from 2.0×10^3 to 3.0×10^5 CFU g⁻¹. The fungal total counts during two months of sampling, were slightly over the limit value of 1×10^5 CFU g⁻¹, which ensure the hygienic quality of the feed. Potentially toxicogenic species presented moderate mean colony counts. Many of the fungi isolated from poultry feeds are mycotoxin producers. Fumonisin had the highest

incidence, and were found in 97 % of the analyzed samples followed by aflatoxin B₁ (46 %), zearalenone (18 %) and deoxynivalenol (6 %). On the co-occurrence of both carcinogenic mycotoxins, all of the FBs contaminated feed samples were co-contaminated with AFB₁. The results show the relevance of the samples screening for viable fungi propagules and the surveillance of their associated mycotoxins in poultry feeds.

Introduction

The contamination of agricultural commodities with fungi able to produce toxic metabolites is often unavoidable and of worldwide concern. These products constitute a rich habitat for fungi because they not only contain enough nutrients, such as carbohydrates, proteins, lipids and other growth factors, but also moisture. Mould contamination of grains occurs during the pre-harvest and/or the post-harvest periods. During these periods, temperature and humidity play an important role in the growth of fungi. The development of modern agricultural methods, together with large scale processing, has exacerbated the problem, resulting in outbreaks of acute diseases called mycotoxicoses and also chronic conditions, often not recognized as such. The correct characterization and identification of food-spoilage mold species is becoming essential in order to control and prevent food contamination by these microorganisms and potential mycotoxin production. Changing agricultural practice can affect the incidence of the infection and the genera present in cereal crops over the years. Dry storage conditions limit the growth of the mycelium, but mycotoxins may be produced by the mycelium present in and on the grain kernels at the moment of harvest (36).

Corn is one of the most commonly cultivated crops in Argentina, and Córdoba province provides about 25 % of the national production. Most of the production is for animal feed, either directly or as part of mixed feed such as poultry feed, and hog feed. Commercial mixed feeds in Argentina are a basic element in modern avian husbandry. Their compositions include mixtures of home grown cereals, mainly corn plus, other additives.

Most of the fungi and mycotoxins present in the cereals are stable under storage and process conditions and will contaminate the final feedstuffs into which they are incorporated. The mixed feed mycological quality need to be controlled because fungal and mycotoxin contamination usually lead to nutrient losses and can have detrimental effects on poultry health and production (35).

The frequent incidence of relatively high toxin levels indicates potential problems in grain-fed animals because of chronic exposure to these toxins. The natural co-occurrence of zearalenone and fumonisins with aflatoxins in corn based feeds has been reported in several countries (2, 10, 14, 23, 30, 31, 32, 41, 42, 43, 45, 46, 56).

Due to the fact that in Argentina there is little information about this topic, the aim of this work was to study the presence and abundance of toxigenic *Aspergillus*, *Penicillium* and *Fusarium* species and the occurrence of mycotoxins in poultry feeds in a feed- manufacturing plant of the south of Córdoba province, Argentina. The results of the mycological and toxicological analysis will be compared with previous

studies carried out with poultry feeds in the same region from years 1995/1996 and 1996/1997 (11, 12). In this way, a representative impression of the mycological and toxicological quality of poultry feed was obtained.

Materials and Methods

Sampling. Poultry feed samples all meant for poultry consumption, were randomly collected from a mixed feed production plant (chosen at random). A total of 120 samples (10 samples per month) were collected from May 1998 to April 1999, in the factory located in the department of Río Cuarto from Córdoba province, Argentina. Samples of 10 kg each were freshly taken during the production line. These primary samples were finely ground in a Buehler laboratory mill and thoroughly mixed before taken aliquots for moisture content, fungal and mycotoxin analysis.

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 portion of each sample with 90 ml of 0.1% peptone water solution. Serial dilutions 10^{-2} until 10^{-4} concentration were made from each material and 0.1 ml aliquots were inoculated by triplicates on two media. Dichloran rose bengal chloramphenicol agar (DRBC) was used for general fungal enumeration (34) and Nash-Snyder medium for isolation of *Fusarium* species (27).

Plates containing medium DRBC were incubated in darkness at 28°C for 7 days in normal atmosphere. 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 contained 10-100 CFU were used for counting and the results were expressed as CFU per gram of sample. Taxonomic identification of the different genera was made according to microscopic criteria in accordance with appropriate keys (3).

Fungal isolation and identification. Fungal colonies identified as *Aspergillus* and *Penicillium* were subcultured in potato dextrose agar (PDA) and *Fusarium* in carnation leaf agar (CLA) for later identification of species. Taxonomic identification of all colonies considered as different was achieved through macroscopic and microscopic studies followed by taxonomic schemes of each particular group of fungi. *Aspergillus* and *Penicillium* species were identified according to taxonomic schemes proposed by Pitt and Hocking (1997), Klich and Pitt (1988), Pitt (1988), and *Fusarium* species according to Nelson *et al*, (1983).

Mycotoxin analysis. A total of 120 samples were analysed for mycotoxins. Aflatoxins and fumonisins analysis were performed by HPLC, following the methodology proposed by Trucksess *et al* (1994) and Shephard *et al* (1990), respectively. Zearalenone and deoxynivalenol analysis were performed by TLC according to AOAC (AOAC, 1995) and Trucksess *et al* (1984), respectively.

Statistical analysis. The analysis of variance on a completely randomized design was used to compare the means between two periods (period 1: May to September/1998, period 2: October/1998 to April/1999) of four variables: total mold count, moisture content, AFB₁ and fumonisins. When needed a variable transformation was performed because the variable was not continuous. For the situations that not verified the assumptions of Variance Analysis Wilcoxon parametric test was used (24, 26, 49). Each data sample (n = 10 per month) was done by triplicate.

Results

The occurrence of fungi genera was defined as the percentage of samples in which each fungus was present. Survey of 120 samples of poultry feeds showed the presence of 15 genera of filamentous fungi (Figure 1). The predominant genera were *Fusarium* spp. and *Penicillium* spp., isolated in 67.5% of the samples, followed by *Aspergillus* spp. (57.5%), among other genus isolated in a minor percentage of the samples.

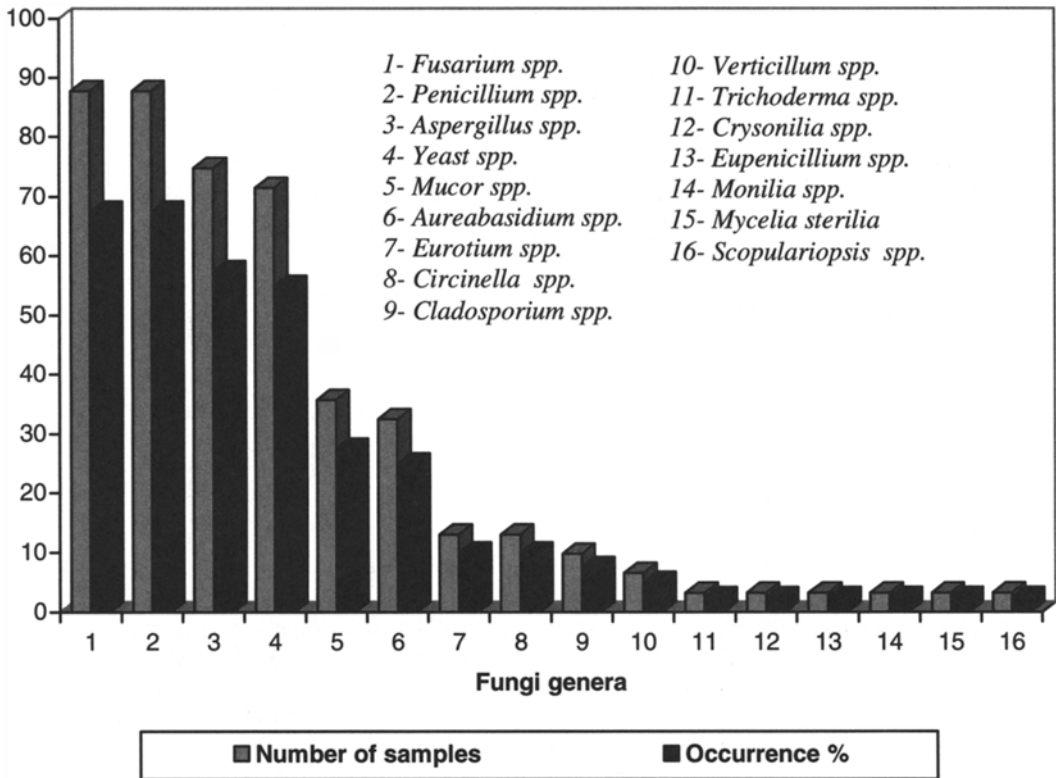


Figure 1 - Distribution of mycoflora in 120 samples of poultry feed samples period 1998/1999.

From the *Aspergillus* and *Eurotium* genera, six and four species respectively were identified. Among *Aspergillus*, *A. flavus* and *A. candidus* were the most frequent. The *Eurotium* species were represented for *E. amstelodami*, *E. chevalieri* and *E. repens* in a low incidence (Figure 2).

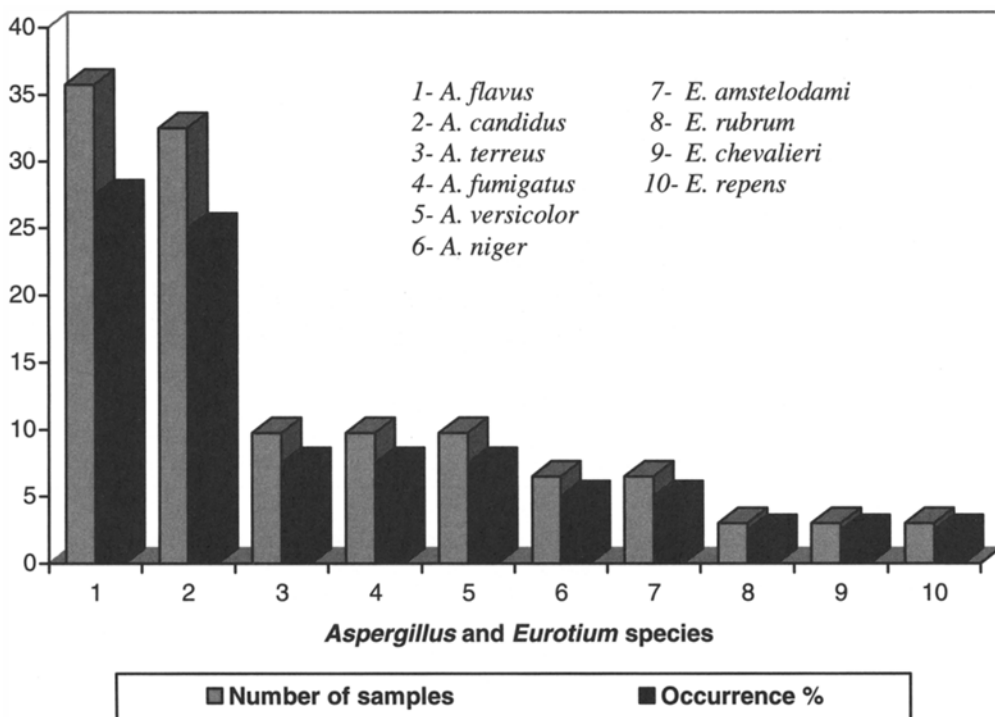


Figure 2 - Distribution of *Aspergillus* species in poultry feed samples period 1998/1999

In the *Penicillium* genus, eleven species were identified and the predominant species were *P. minioluteum*, *P. rugulosum* and *P. implicatum*. In decreasing order *P. crustosum*, *P. purpurogenum* and *P. variable* were isolated among others. Only one teleomorphic species *E. aluteaceum* was isolated (Figure 3).

From the *Fusarium* genus, six species were identified, but *F. verticillioides* and *F. nygamai* were the most frequent (Figure 4).

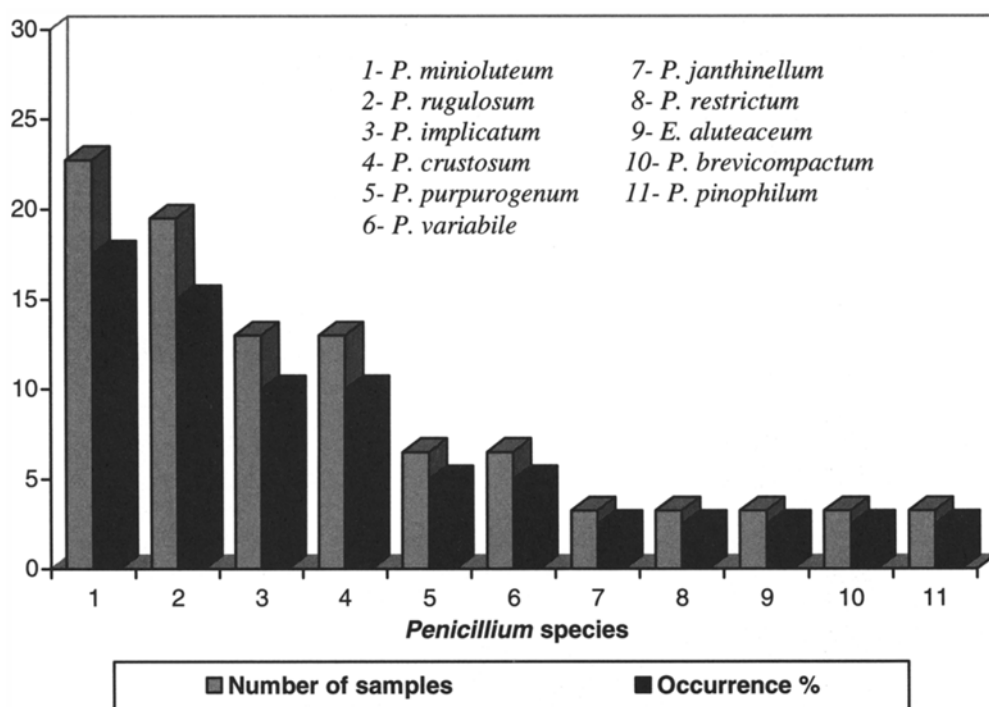


Figure 3 - Distribution of *Penicillium* species in poultry feed samples period 1998/1999.

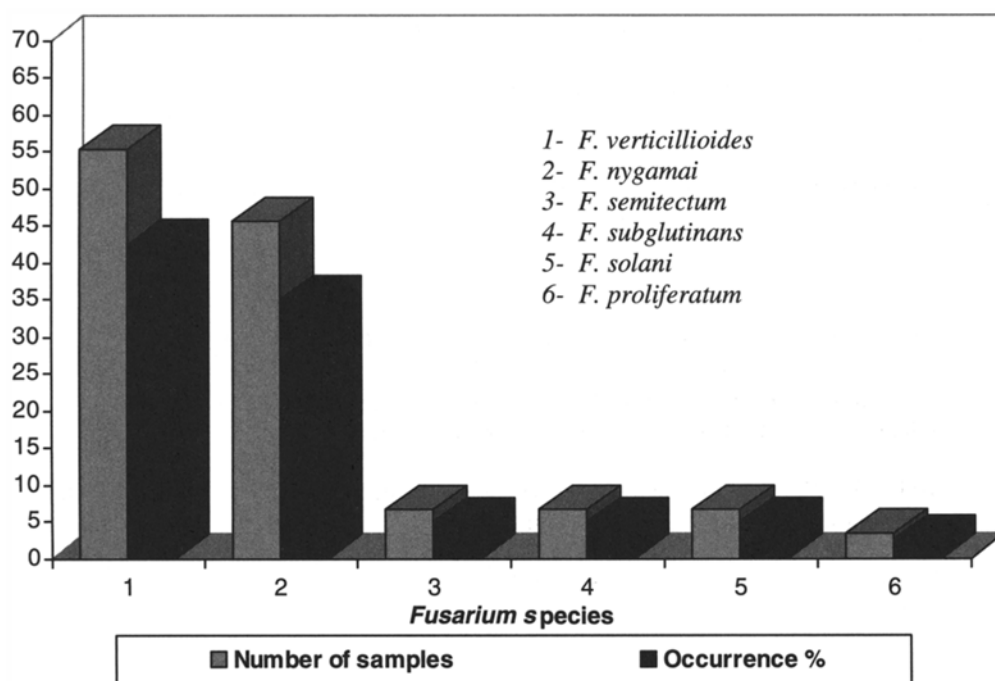


Figure 4 - Distribution of *Fusarium* species in poultry feed samples period 1998/1999.

Yeast, were isolated in high frequency from the most of the samples. The means of fungal total counts in the DRBC medium ranged from 2.0×10^3 to 3.0×10^5 CFU g⁻¹. The highest counts were obtained during the first months (from May to September/1998) and the mean values, from 2.4×10^3 to 3.0×10^5 CFU g⁻¹, were significantly different ($p = 0.013$) from those obtained for the last months, 2.0×10^3 to 7.15×10^4 CFU g⁻¹, of the sampling (October/1998 to April/1999). The moisture content of the samples detected in the first months of sampling were high in relation to the second period ($p = 0.0$). Their percentages ranged from 9.0 to 14.4 % during the twelve months of sampling (Table 1).

Table 1: Mycoflora and moisture content of 120 samples of poultry feeds.

Month	Total mould count ± SE ^a	Range of total count	Moisture content (%) ± SE ^b
May	$2.35 \times 10^5 \pm 4.6 \times 10^4$ ^c	$1.0 \times 10^4 - 6.0 \times 10^5$	14.0 ± 0.01 ^e
June	$2.4 \times 10^3 \pm 5.3 \times 10^2$ ^c	$1.0 \times 10^3 - 8.0 \times 10^3$	14.2 ± 0.12 ^e
July	$3.0 \times 10^5 \pm 8.0 \times 10^4$ ^c	$2.0 \times 10^4 - 1.2 \times 10^6$	14.4 ± 0.23 ^e
August	$1.8 \times 10^4 \pm 4.0 \times 10^3$ ^c	$1.0 \times 10^3 - 4.0 \times 10^4$	12.2 ± 0.03 ^e
September	$3.5 \times 10^4 \pm 1.0 \times 10^4$ ^c	$1.0 \times 10^3 - 3.0 \times 10^5$	10.8 ± 0.04 ^e
October	$4.0 \times 10^4 \pm 1.35 \times 10^4$ ^d	$1.0 \times 10^3 - 3.0 \times 10^5$	10.0 ± 0.06 ^f
November	$2.0 \times 10^3 \pm 3.2 \times 10^2$ ^d	$1.0 \times 10^3 - 5.0 \times 10^3$	9.0 ± 0.03 ^f
December	$3.15 \times 10^3 \pm 6.0 \times 10^2$ ^d	$1.0 \times 10^3 - 1.0 \times 10^4$	9.2 ± 0.16 ^f
January 1999	$6.4 \times 10^4 \pm 2.4 \times 10^4$ ^d	$1.0 \times 10^3 - 4.3 \times 10^5$	9.3 ± 0.05 ^f
February	$7.15 \times 10^4 \pm 2.4 \times 10^4$ ^d	$1.0 \times 10^3 - 4.0 \times 10^5$	9.4 ± 0.07 ^f
March	$2.0 \times 10^3 \pm 2.3 \times 10^2$ ^d	$1.0 \times 10^3 - 5.0 \times 10^3$	10.0 ± 0.42 ^f
April	$3.0 \times 10^3 \pm 8.0 \times 10^2$ ^d	$1.0 \times 10^3 - 2.0 \times 10^4$	10.8 ± 0.53 ^f

^a Mean mould count (CFU g⁻¹). SE: standard error.

^b Mean percentage moisture.

n: 120 samples.

Significative differences were found between ^c and ^d ($p = 0.013$), ^e and ^f ($p = 0.0$)

Potentially toxicogenic species, from 80 out 120 samples chosen at random, presented moderates mean colony counts. *A. flavus* var. *flavus* ranged from 1.0×10^3 to 2.0×10^4 CFU g⁻¹. The fumonisins producers ranged from 1.0×10^3 to 7.0×10^5 CFU g⁻¹. The colony counts of *Penicillium* species ranged from 1.0×10^3 to 2.0×10^5 CFU g⁻¹ (Table 2).

Table 2: Total mould counts of toxicogenic species of *Aspergillus*, *Fusarium*, and *Penicillium* in poultry feeds.

Species	Mould count (CFU g ⁻¹)	
	Range ^a	Mean ± SE ^b
<i>A niger</i>	2.0 x 10 ³ – 1.0 x 10 ⁴	6.0 x 10 ³ ± 4.0 x 10 ³
<i>A flavus</i> var. <i>flavus</i>	1.0 x 10 ³ – 2.0 x 10 ⁴	5.0 x 10 ³ ± 2.0 x 10 ³
<i>A terreus</i>	1.0 x 10 ³ – 1.5 x 10 ³	1.3 x 10 ³ ± 2.5 x 10 ³
<i>A candidus</i>	1.0 x 10 ³ – 1.0 x 10 ⁴	8.0 x 10 ³ ± 3.0 x 10 ³
<i>A fumigatus</i>	1.0 x 10 ⁴ – 2.0 x 10 ⁴	1.5 x 10 ⁴ ± 3.5 x 10 ³
<i>A versicolor</i>	1.0 x 10 ³ – 5.0 x 10 ³	3.0 x 10 ³ ± 8.5 x 10 ²
<i>F verticillioides</i>	1.0 x 10 ³ – 3.0 x 10 ⁵	6.0 x 10 ⁴ ± 2.0 x 10 ⁴
<i>F nygamai</i>	1.0 x 10 ³ – 7.0 x 10 ⁵	5.5 x 10 ⁴ ± 2.0 x 10 ⁴
<i>F subglutinans</i>	1.0 x 10 ⁴ – 7.0 x 10 ⁴	4.0 x 10 ⁴ ± 3.0 x 10 ⁴
<i>F proliferatum</i>	1.0 x 10 ³ – 1.25 x 10 ³	1.0 x 10 ³ ± 1.25 x 10 ²
<i>F semitectum</i>	1.0 x 10 ³ – 1.0 x 10 ⁵	5.05 x 10 ⁴ ± 5.0 x 10 ⁴
<i>F solani</i>	1.0 x 10 ³ – 5.0 x 10 ³	3.0 x 10 ³ ± 1.0 x 10 ³
<i>P rugulosum</i>	1.0 x 10 ³ – 1.0 x 10 ⁵	4.0 x 10 ⁴ ± 1.6 x 10 ⁴
<i>P pinophilum</i>	1.5 x 10 ⁵ – 2.0 x 10 ⁵	1.75 x 10 ⁵ ± 2.5 x 10 ⁴
<i>P crustosum</i>	1.0 x 10 ⁴ – 2.0 x 10 ⁴	1.3 x 10 ⁴ ± 2.5 x 10 ³
<i>P minioluteum</i>	1.0 x 10 ³ – 2.0 x 10 ⁴	1.5 x 10 ⁴ ± 3.5 x 10 ³
<i>P purpurogenum</i>	1.0 x 10 ⁴ – 1.0 x 10 ⁵	5.5 x 10 ⁴ ± 4.5 x 10 ⁴
<i>P variable</i>	1.0 x 10 ³ – 2.0 x 10 ³	1.5 x 10 ³ ± 5.0 x 10 ²
<i>P implicatum</i>	1.3 x 10 ⁴ – 2.0 x 10 ⁴	1.5 x 10 ⁴ ± 2.0 x 10 ³
<i>P janthinellum</i>	1.0 x 10 ⁵ – 1.25 x 10 ⁵	1.0 x 10 ⁵ ± 1.25 x 10 ⁴
<i>P restrictum</i>	2.5 x 10 ⁴ – 2.0 x 10 ⁴	2.5 x 10 ⁴ ± 2.0 x 10 ³
<i>P brevicompactum</i>	1.0 x 10 ³ – 1.25 x 10 ³	1.0 x 10 ³ ± 1.25 x 10 ²

^a Limit values of fungal counts (CFU g⁻¹) for each specie. SE: standard error.

^b Mean values of fungal counts (CFU g⁻¹) for each specie.
n: 80 samples.

Among the three groups of analyzed mycotoxins, fumonisins had the highest incidence, and were found in 126 (97%) of the analyzed samples, followed by AFB₁ present in 60 samples (46%).

The levels of fumonisins varied from 136 to 4,270 ng/g for FB₁ (mean concentration 1,834 ng/g), from 39 to 1,710 ng/g for FB₂ (mean concentration 676.5 ng/g) and from 28 to 955 ng/g for FB₃ (mean concentration 278 ng/g). Total fumonisins concentration ranged from 312 to 5,727 ng/g with average value of 2,789 ng/g. The FB₂/FB₁ ratio ranged from 0.14 to 0.84 (mean value 0.42 ng/g) and FB₃/FB₁ ratio ranged from 0.07 to 0.57 (mean value 0.20 ng/g). The mean values of FB₁, FB₂ and FB₃ obtained in the first period of sampling were not significantly different from those obtained in the last period (p = 0.1298, 0.8144, 0.9560 respectively) (Table 3).

Table 3: Fumonisin analysis of 120 samples of poultry feeds.

Month	% positive samples ^a	Fumonisin levels (ng/g) $\pm$ SE ^b				
		FB ₁	FB ₂	FB ₃	Totals fumonisins	FB ₂ /F B ₁ FB ₃ /F B ₁
May	100	2024.5 $\pm$ 5.53	841.5 $\pm$ 2.05	208.5 $\pm$ 4.3	3074.5	0.41 0.10
June	100	1214 $\pm$ 9.85	614 $\pm$ 9.7	215.5 $\pm$ 2.3	2044	0.50 0.18
July	100	2814 $\pm$ 6.93	663 $\pm$ 1.6	253 $\pm$ 1.2	3730	0.23 0.09
August	100	4270 $\pm$ 135.0	725 $\pm$ 2.9	292 $\pm$ 6.03	5287	0.17 0.07
September	100	3154 $\pm$ 23.6	525 $\pm$ 13.5	227 $\pm$ 8.82	3905.5	0.17 0.07
October	100	136 $\pm$ 3.51	114.5 $\pm$ 2.94	63 $\pm$ 1.6	313.5	0.84 0.46
November	100	181 $\pm$ 2.08	111.5 $\pm$ 5.92	72.5 $\pm$ 1.25	365	0.61 0.40
December	100	3517 $\pm$ 16.8	1710 $\pm$ 15.1	500 $\pm$ 5.8	5727	0.48 0.14
January	100	1665 $\pm$ 32.4	1326 $\pm$ 14.2	955 $\pm$ 27.4	3946	0.80 0.57
February	80	2591 $\pm$ 21.5	1537 $\pm$ 31.8	550 $\pm$ 45.1	4678	0.59 0.21
March	80	273 $\pm$ 3.30	38.5 $\pm$ 0.76	27.5 $\pm$ 3.71	339.5	0.14 0.09
April	100	230.5 $\pm$ 5.80	41 $\pm$ 0.35	39.5 $\pm$ 2.95	311	0.18 0.17

^a Percentage of positive samples from ten samples for each month.^b Mean of concentration mycotoxin based on positive samples only. SE: Standard error.

ND: not detected.

n: 120 samples.

Detection limits FBs (ng/g): 20

The levels of AFB₁ varied from 14 to 174 ng/g (mean concentration 80 ng/g) during the sampling period. Zearalenone was detected in 23 (17.7%) of the samples with levels ranging from 1,550 to 4,507 ng/g (mean concentration 2,581 ng/g). Only 8 samples (6.15%) contained DON with a mean level of 124 ng/g.

On the co-occurrence of both carcinogenic mycotoxins, all of the FBs contaminated feed samples were co-contaminated with AFB₁. The highest of AFB₁ contaminated feed (174 ng/g) were contaminated with FB₁ at a concentrations of 3,154 ng/g. The feed samples contaminated with the highest levels of FB₁ (4,270 ng/g) were co-contaminated with AFB₁ at a concentrations of 40 ng/g.

No significant differences were detected in the levels of AFB₁ between the period of sampling ($p = 0.8836$) but ZEA and DON were not analyzed statistically because there were a high percentage of negative samples (Table 4).

The results were corrected by recovery rates. The recovery rates of the methodology used for mycotoxins detection ($n=4$) were 86% for AFB₁, ZEA and DON 99% and 84%, respectively and for fumonisins 90%, 87%, 83% for FB₁, FB₂ and FB₃, respectively.

Discussion

Our results showed that *Fusarium*, *Penicillium* and *Aspergillus* genera were the most prevalent from poultry feeds in the sampling period. This information confirms the results obtained in previous studies with samples collected from the same industry and analyzed following the same methodology (11, 12). These genera were also found in commercial corn meal in Argentina (15) and by other authors in corn-based feed in Spain (1, 7, 8, 9).

Many of the fungi isolated from poultry feeds are capable of producing mycotoxins; others may be associated with mycoses or allergenic responses, while others with spoilage. In general, all species found in this study have also been isolated by other workers from mixed feeds and feed components (17, 18, 30, 38). The significance of yeast, which was frequently isolated from most of the samples, is not known. The *Aspergillus* species, *A. versicolor*, *A. ochraceus*, *A. candidus*, *A. terreus*, *A. niger*, are often associated with both freshly harvested and storage cereals (36). Some of the species are capable of producing mycotoxins including sterigmatocystin, citrinin, ochratoxins among others. Various mycotoxicoses have been attributed to *A. fumigatus*, and there is little evidence of the natural occurrence of its toxins particularly associated with heating of cereals storage (20, 30, 55).

Our results showed that the *Penicillium* species isolated from poultry feeds were characteristic of those isolated from cereal grains such as corn, rice and wheat (17). Several species, *P. citrinum*, *P. glabrum* and *P. purpurogenum* are capable of producing mycotoxins (34). Other nephrotoxicogenic metabolites produced by *Penicillium* species, naphthoquinones, xanthomegnin, viomellein and vioxantin are sometimes found together with ochratoxin A and citrinin (40). In a previous study many of the *Aspergillus* and *Penicillium* species isolated in this work were found with different frequency of isolation (21). In a work with dwarf kernels samples in Brazil, the most frequent isolated species were: *P. brevicompactum* and *P. glabrum* (17).

Table 4: Mycotoxins analysis from 120 samples of poultry feeds.

Month	Mycotoxin levels					
	AFB ₁			ZEA		DON
	% positive samples ^a	Levels (ng/g) ± SE ^b	% positive samples ^a	Levels (ng/g) ± SE ^b	% positive samples ^a	Levels (ng/g) ± SE ^b
May	0	ND	0	ND	0	ND
June	0	ND	100	4507 ± 146	80	124 ± 5.55
July	80	27 ± 2.5	0	ND	0	ND
August	80	40 ± 4.6	0	ND	0	ND
September	100	174 ± 16.9	80	1687 ± 27.8	0	ND
October	50	92 ± 3.75	0	ND	0	ND
November	50	132 ± 2.5	0	ND	0	ND
December	0	ND	0	ND	0	ND
January	60	42 ± 1.6	0	ND	0	ND
February	0	ND	0	ND	0	ND
March	70	14 ± 1.35	0	ND	0	ND
April	60	91 ± 2.7	50	1550 ± 20.5	0	ND

^a Percentage of positive samples from ten samples for each month.
^b Mean of concentration mycotoxin based on positive samples only. SE: Standard error
ND: not detected.
n: 120 samples.
Detection limits (ng/g) AFB₁ =2, ZEA=50, DON=100.

The fungal total counts during two months of sampling (May and July), were slightly over the value that was proposed as limit (1×10^5 CFU g⁻¹) to ensure the hygienic quality of this feed. In general the fungal counts detected during this period of sampling were moderate compared with those obtained in our previous study (11, 12). The screening of samples for viable fungal propagules is a useful exercise in itself as an indicator of mould contamination but it also complements the analysis of mycotoxins that could be present. In this study high incidence of *F. verticillioides*, *F. nygamai* and *A. flavus* with moderate mean individual counts was observed. In Argentina, these species were isolated in the highest frequency from corn meal in Córdoba province (15, 16).

Nepote *et al* (1997) obtained 63% of corn samples containing *A. flavus* with counts of 10^3 CFU g⁻¹ in corn from Santa Fé. In South Africa, Dutton and Kinsey (1995), observed samples of feed companies containing 106 propagules/g of *Fusarium* samples spp, mainly *F. verticillioides* with 69% of contaminated samples.

In this study AFB₁ contaminated the highest number of samples (60%). Although *A. flavus* was isolated in high frequency, the toxin levels detected were low. This data agree with those obtained previously (11, 12).

The incidence of ZEA in several samples would indicate the presence of other species of *Fusarium* normally associated with high production of this toxin. *F. graminearum* group 1 has been reported to produce high levels of ZEA in wheat, corn and barley straw (5, 25). These species were isolated in a previous study and were found in a low number of samples that contained important levels of toxin (11). The low number of contaminated samples and low toxin levels found suggest that the incidence of ZEA in poultry feeds of Argentina varies from year to year, and prevalence seems to be linked to drought conditions during the maize growing season.

As regards DON, there was a poor incidence of this toxin. Zearalenone and DON have already been reported in corn in Argentina at similar levels as informed in poultry feeds samples. While in wheat and pig feeds high levels of these toxins were found (4, 22, 52, 53). By contrast none of corn meal samples contained detectable amount of aflatoxins, ZEA and DON (15).

The occurrence of fumonisins is a preharvest and harvest problem, if there is rainfall during corn harvest, favorable conditions will be created for their production. Thus, these toxins might go on during the storage period and be part raw material of feedstuffs.

A survey carried out in some European countries such as Italy, France and Spain has shown that *F. verticillioides* is the most frequently isolated fungal species from maize in the field, harvested maize and maize-based commodities (6, 13, 39). Our results agree with these authors.

Previous surveys for mycotoxins in animal feed and food ingredients in Argentina have shown the presence of fumonisins in corn-foods and feed. Solovey *et al* (1999) observed that 95 % of samples of corn foods were contaminated with FB₁, FB₂ and FB₃. Ramírez *et al* (1996) showed that in commercial hybrid corns, more than 60% of the samples contained fumonisins in levels higher than 1,000 ng/g but only 5 samples (10%) were contaminated with levels above 5,000 ng/g. Farnochi (2000), observed 40% of corn meal samples were contaminated with FB₁ and FB₂ at levels ranging from 44 to 2,000 ng/g. The average levels of these toxins were similar to the ones detected in our survey of poultry feeds.

In other countries the simultaneous occurrence of trichothecenes, ZEA, fumonisins and aflatoxins in corn and feeds was reported (2, 14, 42, 45, 47). Earlier reports demonstrated high prevalence of fumonisins and aflatoxins in corn based feeds and feedstuffs (2, 14, 45, 46). Ali *et al* (1998) reported that all of the aflatoxin contaminated samples were co-contaminated with fumonisins. The co-occurrence of DON, FB₁, FB₂, FB₃, and ZEA were detected in corn collected from Korea (47). Scudamore *et al* (1998) screening maize products (germ, gluten, meal, bran) used in the elaboration of animal feed in United Kingdom, encountered many samples containing a multi-toxin mixture of trichothecenes, fumonisins and moniliformin. The highest contaminated samples of maize and maize meal contained a mixture of ZEA, DON, nivalenol, FB₁, FB₂ and moniliformin. Fumonisins occurred in all screened samples. In these samples, the aflatoxins and ochratoxin A were encountered in low levels.

These studies have indicated that *Fusarium* mycotoxins occur widely in feeds in combination with aflatoxins, ZEA and trichothecenes sometimes at high levels. Present results agree with earlier studies on incidence of mycotoxins in poultry feeds in Argentina because fumonisins and AFB₁ were found to co-occur in 60% of the feedstuff samples.

The absence of mycotoxins in some poultry feeds samples suggests that there are seasonal or annual variations in AFB₁, ZEA and DON contamination of corn kernels used for feed elaboration. Therefore, there is need for constant surveillance of grains in storage silos to determine the levels of these mycotoxins. From the results obtained here and those reported by other authors, the regulation of fumonisins levels in maize and its products requires urgent attention by regulatory organizations.

As a consequence of the raw material is constantly arriving to the feed production plant from different storehouses, which cover a wide corn producer region (south of Córdoba province, Argentina), a correlation between fungi/mycotoxins in feedstuffs and raw materials over the year should be further investigated.

Acknowledgments

The authors are grateful to the SECyT (Secretaría de Ciencia y Técnica, Universidad Nacional de Río Cuarto) and CONICOR (Consejo de Investigaciones de la Pcia. De Córdoba) which supported this research through grants, Res. N° 241/99 and 1655/99 respectively. CM Thanks to CONICOR for a research fellowship. SMC holds a research position at Consejo Nacional de Ciencia y Tecnología (CONICET).

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Manuscript received March 21, 2002; accepted Sept. 23, 2002.