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Surveillance of Toxigenic Fungi and Ochratoxin A in Feedstuffs from Córdoba Province, Argentina

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ABSTRACT

The aim of this work was to evaluate the incidence of potential ochratoxigenic mycoflora and ochratoxin A (OA) in poultry, pig and rabbit feeds. Eighty poultry, pig and rabbit feed samples were taken at random from factories located from Córdoba province, Argentina, over a period of 8 months. Isolation and quantitative enumeration of fungal propagules were done on DRBC and DG18 media. The predominant species were *A. candidus*, *A. flavus*, *A. terreus*, *A. parasiticus*, *P. implicatum P. minioluteum*, *P. crustosum* and *P. citrionigrum*. The distribution of section *Nigri* species varied according to the feedstuffs analysed. The frequency of *A. niger* var. *niger* was noticeably high in poultry feed samples on DRBC medium. The *Nigri* section species was present at moderate mean colony counts (CFU/g) from three feeds. Mycotoxin analysis of these samples showed that OA was detected in 15%, 10% and 12% of pig, poultry and rabbit feed samples, respectively. The mean levels detected ranged between 15 and 25 ng/g from three feeds. The presence of ochratoxigenic species of *Nigri* section and OA in feeds indicates the risk of potential exposure of poultry, pigs and rabbits through the ingestion of feeds.

Keywords: *Aspergillus* section *Nigri, Aspergillus ochraceus*, *Penicillium verrucosum,* ochratoxin A, poultry feed, pig feed, rabbit feed

Abbreviations: CFU, colony-forming unit; DG18, dichloran–18% glycerol agar; DRBC, dichloran–rose Bengal–chloramphenicol agar; HPLC, high-pressure liquid chromatography; OA, ochratoxin; PDA, potato dextrose agar

INTRODUCTION

Most cereals, grains and feed derivatives are prone to fungal growth during production, processing, transportation and storage (Frisvad and Samson, 1992). Mycotoxins are primarily found in agricultural crops such as cereals and oilseeds as well as products derived from them (Beardall and Miller, 1994). The relatively high intake of raw materials in the diet of livestock such as poultry, pigs and rabbits can have adverse effects on animal health and on productivity when mycotoxin-contaminated feeds are

consumed (Smith *et al.*, 1995). The ubiquity of potentially ochratoxigenic moulds and the large numbers of cereals and foods and feed-based cereals in which the natural occurrence of ochratoxin A (OA) has been reported (Jørgensen *et al.*, 1996; Jørgensen, 1998; Trucksess *et al.*, 1999; Rafai *et al.*, 2000; Scudamore and Patel, 2000, Vrabcheva *et al.*, 2000) led many European countries to introduce legislation aimed at limiting and controlling exposure (Hohler, 1998). OA has been shown to be nephrotoxic in monogastric animals such as pigs and poultry, carcinogenic in kidney, teratogenic to central nervous system and to have immunosuppressive effects in laboratory animals (NTP, 1989; Kuiper Goodman and Scott, 1996; Stoev *et al.*, 1998). The presence of OA in pig and poultry feeds also raises some concerns for the livestock industry. Animals consuming OA have decreased growth rates (Elling, 1983; Mortensen *et al.*, 1983; Gentles *et al.*, 1999) and may be more susceptible to subclinical intoxications (Harvey *et al.*, 1992; Gremmels *et al.*, 1995). The decrease in productivity may result in important losses to the livestock industry (Stoev *et al.*, 1998; Ferrufino-Guardia *et al.*, 2000).

Ochratoxigenic species can be found in two fungal genera, but the genus *Aspergillus* is undoubtedly one of the most important for the number of potential producers. These species are reported in the *Circumdati* section (*A. ochraceus*, *A. melleus*, *A. alliaceus*, *A. petrakii*, *A. ostianus*, *A. sclerotiorum*, *A. sulphureus*, *A. auricomus* and *A. albertenses*) (Varga *et al.*, 1996) and are not common contaminants in agricultural commodities. More recently, the number of reports dealing with the production of OA by members of the *Nigri* section has been increasing (Abarca *et al.*, 2001). According to previous mycological surveys, *A. niger* is one of the most frequently isolated species from agricultural commodities (Bucheli *et al.*, 1998; Dalcero *et al.*, 1998; Magnoli *et al.*, 1998; Da Rocha Rosa *et al.*, 2002). It is therefore reasonable to suspect that black aspergilli may be an important source of OA in tropical and subtropical zones. OA production by *A. niger* and OA content of feeds in Argentina were outlined in a previous report (Dalcero *et al.*, 2002). Only a few studies have integrated the incidence of OA in foods with production by specific fungi, and such studies have mainly focused on *A. ochraceus* and *P. verrucosum* (Pitt and Hocking, 1997).

We have been able to demonstrate the presence in feedstuffs of many mycotoxins of toxicological importance, such as aflatoxins, deoxynivalenol, fumonisins and zearalenone, that strongly affect animal production and health (Dalcero *et al.*, 1997, 1998; Magnoli *et al.*, 1998, 1999, 2002). In Argentina there is no available information on the natural occurrence of ochratoxigenic fungi in feedstuffs. Dalcero and colleagues (2002) reported OA incidence and *Aspergillu*s section *Nigri* species and its capacity to produce this mycotoxin in feedstuffs. The aim of this work was to continue this research, for which it became necessary to determine whether OA origin was due only to the presence of *Aspergillus* section *Nigri* species or whether other *Aspergillus or Penicillium* species, important producers of OA, could be identified from poultry, pig and rabbit feeds.

MATERIALS AND METHODS

Sampling

Eighty poultry, pig and rabbit feed samples were taken at random from factories located in Córdoba province, Argentina, over a period of 8 months. For each feed, 10 samples per month were chosen. Samples of 10 kg each were taken from the production line. These primary samples were finely ground in a Buehler laboratory mill and thoroughly mixed before aliquots were taken for fungal and ochratoxin analysis.

Feed composition

Pelleted poultry and pig feeds contain 60% corn. Corn was replaced by alfalfa, oat and barley in rabbit feed; and according to market availability, the protein value was achieved by adding sunflower pellets or soy. Vitamins or growth promoters, essential amino acids such as lysine and methionine, and coccidiostats were added to all the poultry, pig and rabbit feed.

The raw materials used in the manufacturing of each feed are constantly arriving at the production plant from different storehouses located in a wide cereal-producing region.

Mycoflora determination

Isolation and quantitative enumeration of fungal propagules were done on solid media using the surface-spread method by blending a 10 g portion of each sample with 90 ml of 0.1% peptone water solution. Serial dilutions to 10^{-4} concentration were made from each material and 0.1 ml aliquots were inoculated in triplicate for fungal enumeration on dichloran–rose Bengal–chloramphenicol agar (DRBC) and dichloran–18% glycerol agar (DG18) (Pitt and Hocking, 1997). The plates were incubated in darkness at 28°C for 7 days in a normal atmosphere. On the last day of incubation, plates that contained only 10–100 colony-forming units (CFU) were used for counting and the results were expressed as CFU per gram of sample. Taxonomic identification of the *Aspergillus* and *Penicillium* genera was made according to microscopic criteria in accordance with appropriate keys (Pitt and Hocking, 1997).

Aspergillus *and* Penicillium *isolation and identification*

Fungal colonies identified as *Aspergillus* and *Penicillium* were subcultured on potato dextrose agar (PDA) for later identification at species level. Taxonomic identification of all colonies considered different was achieved through macroscopic and microscopic studies followed by standard tests, which were related to the genera of each particular

fungal group. *Aspergillus* and *Penicillium* species were identified according to taxonomic schemes proposed by Klich and Pitt (1994), Pitt (1988) and Pitt and Hocking (1997).

Ochratoxin A determination in feeds

The detection of OA in 32 samples for each feed was performed by HPLC, following the methodology proposed by Scudamore and MacDonald (1998), with some modifications (Dalcero *et al*., 2002).

Statistical analysis

Analysis of variance was performed on transformed log data and Tukey's *a posteriori* test was applied to compare the means of total mould count for each feed and medium used (Steel and Torrie, 1985). The homogeneity proportions chi- square test was used to determine differences in frequency of infection by section *Nigri* species between the DRBC and DG18 media and three feeds (Agresti, 1990).

RESULTS

The occurrence of fungi was defined as the percentage of the 80 samples in which each fungus was present. Mycological survey of three feeds, using DRBC and DG18 media, showed the presence of a great variety of *Aspergillus* and *Penicillium* species. In general, the percentage of each isolated species depended on the culture medium used. In the genus *Penicillium*, 19 species were isolated from the total of samples. *P. implicatum*, *P. minioluteum* and *P. crustosum* (5–55%) were the predominant species on DG18, and *P. implicatum*, *P. minioluteum* and *P. citrionigrum* (10–30%) were predominant on DRBC (Figures 1, 2 and 3). Fourteen species of *Aspergillus* were identified. The predominant species isolated were *A. candidus* (30–40%), *A. flavus* (30–35%) and *A. terreus* (15–32%), from poultry and pig feeds, while *A. flavus* (15–30%) and *A. parasiticus* (12–17%) were isolated at a higher frequency from rabbit feeds. The distribution of section *Nigri* species varied according to the feedstuffs analysed. Although *A. carbonarius* has been cited as an important OA producer it was not isolated from such substrates. In general, the percentage of potentially ochratoxigenic species showed differences according to the culture media in which the isolation was done. From poultry feeds, *A. niger* var. *niger* was isolated at frequencies of 12.5% and 27.5% (Figure 4). In pig and rabbit feeds, this species was present at 7.5% and 10% (Figure 5 and 6). The species *A. niger* var. *awamori* was isolated from 2.5–17.5% of poultry, pig and rabbit feeds, and *A. japonicus* var. *japonicus* from 2.5–5% of the three feeds. Other species of this section, *A. foetidus* and *A. japonicus* var. *aculeatus* (2.5%), were isolated only from poultry feeds. The frequency of *A. niger* var. *niger* was noticeably high in poultry feed samples on DRBC medium ($p = 0.024$). Significant

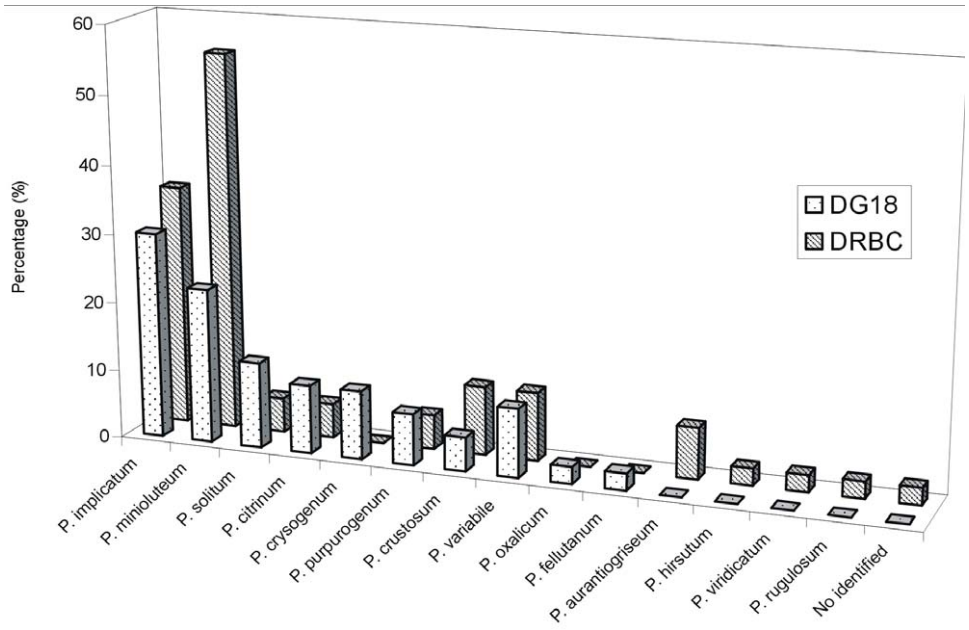

Figure 1. Percentage of *Penicillium* species in poultry feed samples collected during 1999–2000

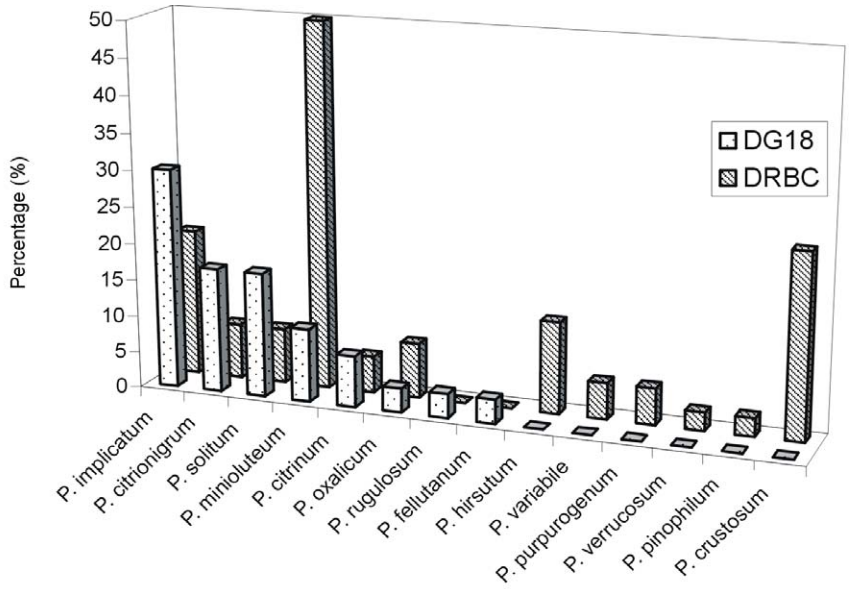

Figure 2. Percentage of *Penicillium* species in pig feed samples collected during 1999–2000

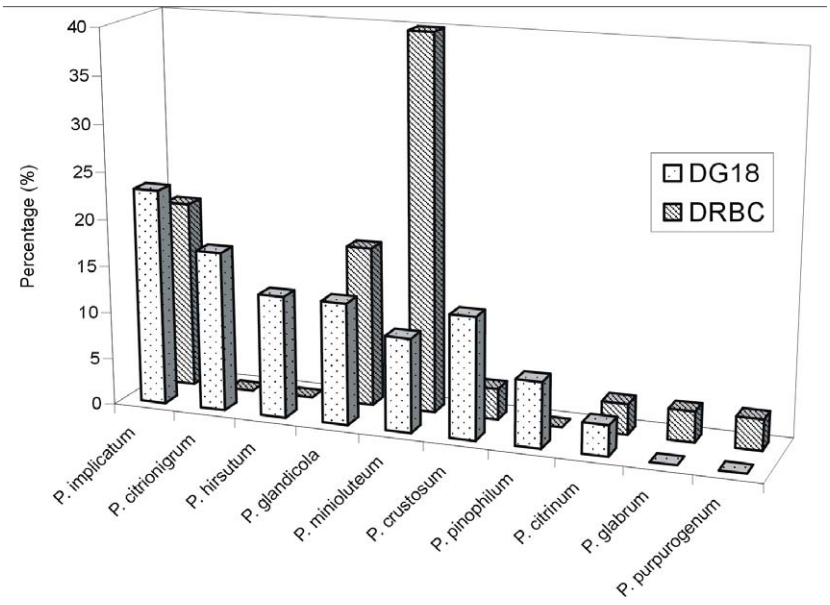

Figure 3. Percentage of *Penicillium* species in rabbit feed samples collected during 1999–2000

differences in *A. niger* var. *awamori* frequency between the feeds (poultry, $p = 0.305$; pigs, $p = 0.176$; rabbits, $p = 1$) and media (DRBC, $p = 0.061$; DG18, $p = 1$) were not detected. The frequency of the other species of *Nigri* section was not analysed statistically because of the low numbers of contaminated samples.

From the *Circumdati* section, *A. ochraceus* was present in low numbers of samples (2.5%) from poultry feeds (Figure 4). Likewise, in the *Penicillium* genus, the potentially ochratoxicogenic species *P. verrucosum* (2.5%) was isolated from pig feeds (Figure 2). Fungal total counts on the DRBC medium from the three feeds ranged from 2×10^2 to 7.5×10^5 CFU/g, and on DG18 medium ranged from 1×10^2 to 8×10^5 CFU/g. The highest fungal counts (10^5 CFU/g), were obtained from three feeds. From the total feed samples, no significant differences were detected in mean total mould counts between DRBC and DG18 media ($p = 0.50$). In relation to the mean total mould counts, significant differences between poultry and rabbit feeds ($p < 0.001$) and between pig and rabbit feeds ($p < 0.001$) were found (Table I, II and III). The *Nigri* section species presented moderate mean colony counts from three feeds: *A. niger* var. *niger* ranged from 1×10^2 to 3.4×10^3 CFU/g; *A. niger* var. *awamori* ranged from 1×10^2 to 4×10^3 CFU/g; *A. foetidus* occurred at 2×10^2 CFU/g; *A. japonicus* var. *aculeatus* ranged from 1×10^2 to 2×10^2 CFU/g; and *A. japonicus* var. *japonicus* ranged from 1×10^2 to 1×10^3 CFU/g. The mean values of counts for *A. ochraceus* and *P. verrucosum* were 10^2 CFU/g (Table IV). Mycotoxin analysis of these samples showed that OA was detected in 15%,

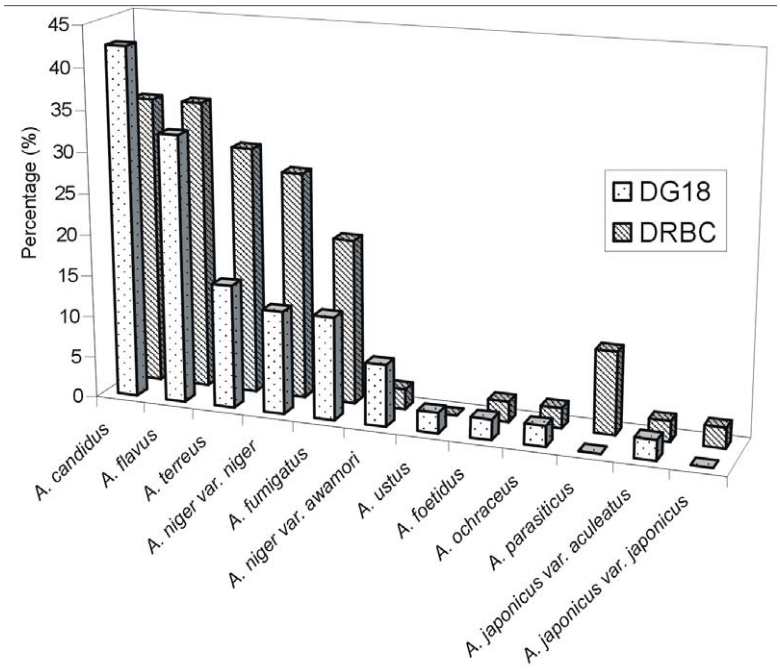

Figure 4. Percentage of *Aspergillus* species in poultry feed samples collected during 1999–2000

10% and 12%, respectively, of pig, poultry and rabbit feed samples. The mean levels detected ranged from 15 to 25 ng/g from three feeds. Recovery of the method was 93%, 95% and 96% from rabbit, pig and poultry feeds, respectively.

DISCUSSION

In general, *Aspergillus* species isolated were similar, with variations not only in frequency but also in counts from each particular feed. The species associated with OA production, *P. verrucosum* and *A. ochraceus*, had not previously been isolated from poultry feeds in our region (Dalcero *et al.*, 1998). However, the results obtained in the present study showed that these species may occur in feedstuffs with low frequency. *A. carbonarius* was not isolated from feedstuffs; this agrees with earlier studies (Dalcero *et al.*, 2002). *Aspergillus* section *Nigri* species are found with a moderate prevalence in this substrate. Most of the isolated *Penicillium* species had been reported previously in cereal grains such as corn, rice and wheat used in food and feed formulations (Abarca *et al.*, 1994; Pitt and Hocking, 1997; Etcheverry *et al.*, 1999; Freire *et al.*, 1999). Many of the *Penicillium* species found (*P. citrinum*, *P. rugulosum*, *P. crustosum*, *P. variabile*, *P. aurantiogriseum* and *P. purpurogenum*) can produce a very wide range of toxic

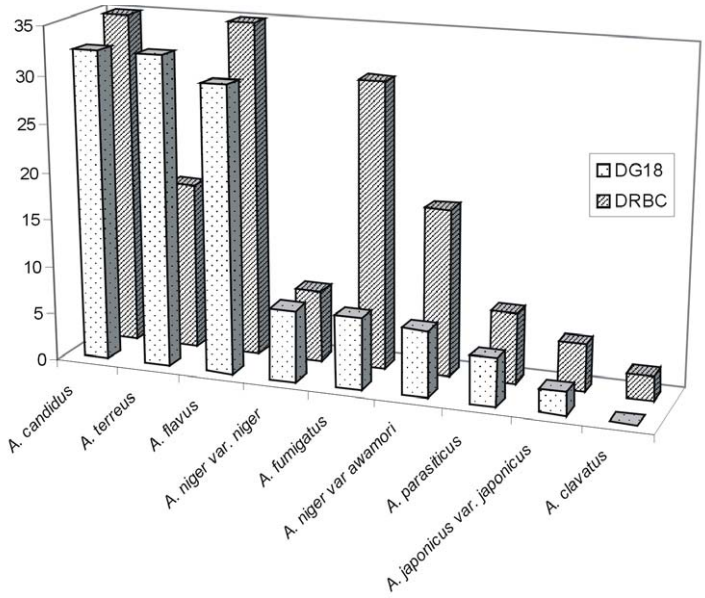

Figure 5. Percentage of *Aspergillus* species in pig feed samples collected during 1999–2000

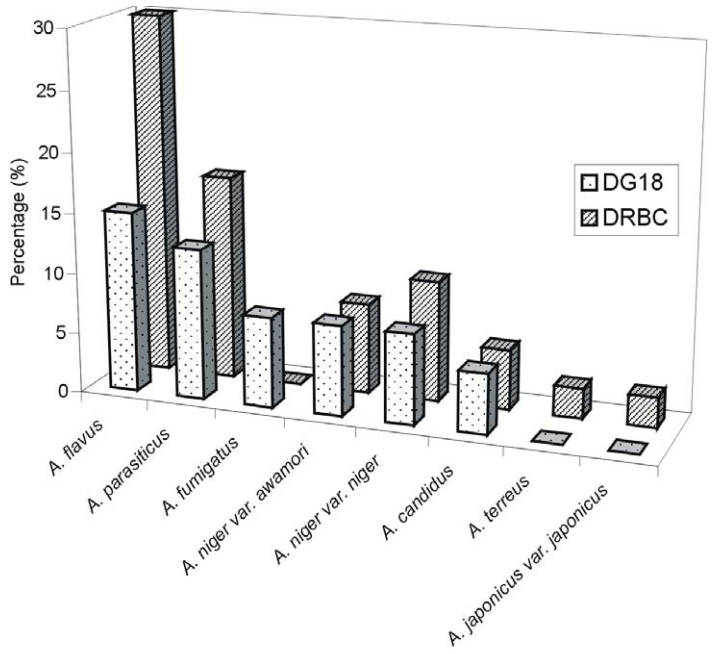

Figure 6. Percentage of *Aspergillus* species in rabbit feed samples collected during 1999–2000

TABLE I
Mycoflora of poultry feeds on DRBC and DG18 media

Month	DRBC (mould count ± SD)			DG18 (mould count ± SD)		
	Total[a]	*Aspergillus*[b]	*Penicillium*[b]	Total[a]	*Aspergillus*[b]	*Penicillium*[b]
May	$2 \times 10^2 \pm 1 \times 10^2$	ND	$6.0 \times 10^2 \pm 4.5 \times 10^2$	$1 \times 10^2 \pm 1 \times 10^2$	ND	$1 \times 10^2 \pm 1 \times 10^2$
July	$3 \times 10^5 \pm 1 \times 10^5$	$5 \times 10^3 \pm 4 \times 10^3$	$4.0 \times 10^4 \pm 2.5 \times 10^4$	$2 \times 10^5 \pm 6 \times 10^4$	$9 \times 10^3 \pm 9 \times 10^3$	$2.5 \times 10^4 \pm 1.0 \times 10^4$
Sept.	$2 \times 10^5 \pm 1 \times 10^5$	$1 \times 10^5 \pm 1 \times 10^5$	$2 \times 10^8 \pm 3 \times 108$	$4 \times 10^5 \pm 6 \times 10^5$	$2 \times 10^5 \pm 4 \times 10^5$	$4 \times 10^3 \pm 2 \times 10^3$
Nov.	$1.6 \times 10^5 \pm 1.3 \times 10^5$	$5.3 \times 10^3 \pm 8.5 \times 10^3$	$8.0 \times 10^3 \pm 1.3 \times 10^4$	$2.0 \times 10^5 \pm 1.4 \times 10^4$	$1.3 \times 10^3 \pm 1.7 \times 10^3$	$6.0 \times 10^3 \pm 3.5 \times 10^3$
Dec.	$5.4 \times 10^4 \pm 3.1 \times 10^4$	$8.2 \times 10^2 \pm 4.0 \times 10^2$	$1.0 \times 10^4 \pm 2.0 \times 10^4$	$5.0 \times 10^4 \pm 2.4 \times 10^4$	$1.0 \times 10^3 \pm 0.0$	$7.5 \times 10^3 \pm 7.7 \times 10^3$
Jan.	$1.0 \times 10^4 \pm 2.0 \times 10^4$	$2.4 \times 10^2 \pm 1.15 \times 10^2$	$5.0 \times 10^2 \pm 5.4 \times 10^2$	$1.0 \times 10^4 \pm 3.6 \times 10^3$	$2.1 \times 10^2 \pm 1.2 \times 10^2$	$1.1 \times 10^3 \pm 2.2 \times 10^3$
Mar.	$2.0 \times 10^5 \pm 1.5 \times 10^5$	$1.3 \times 10^3 \pm 5.8 \times 10^2$	$7.6 \times 10^4 \pm 1.4 \times 10^5$	$5.3 \times 10^4 \pm 3.5 \times 10^4$	$7.0 \times 10^3 \pm 1.2 \times 10^4$	$4.0 \times 10^3 \pm 0.0$
Apr.	$4.0 \times 10^3 \pm 6.0 \times 10^2$	$1.0 \times 10^3 \pm 2.8 \times 10^2$	$2.5 \times 10^2 \pm 3.2 \times 10^2$	$4.0 \times 10^3 \pm 8.0 \times 10^2$	$1.5 \times 10^3 \pm 80.6$	$1.0 \times 10^2 \pm 0.0$

[a]Mean total mould count (CFU/g), $n = 80$

[b]Mean count of *Aspergillus* and *Penicillium* genera (CFU/g)

SD, standard deviation; ND, not detected

TABLE II
Mycoflora of pig feeds on DRBC and DG18 media

Month	DRBC (mould count ± SD)			DG18 (mould count ± SD)		
	Total[a]	*Aspergillus*[b]	*Penicillium*[b]	Total[a]	*Aspergillus*[b]	*Penicillium*[b]
May	$3\times10^{2} \pm 2\times10^{2}$	ND	$5\times10^{2} \pm 4\times10^{2}$	$2\times10^{3} \pm 2\times10^{3}$	ND	$3\times10^{3} \pm 4\times10^{3}$
July	$6\times10^{4} \pm 7\times10^{3}$	$2\times10^{3} \pm 2\times10^{3}$	$3.0\times10^{3} \pm 3.5\times10^{3}$	$4\times10^{4} \pm 2\times10^{4}$	$2\times10^{3} \pm 1\times10^{3}$	ND
Sept.	$2\times10^{4} \pm 1\times10^{4}$	$7\times10^{2} \pm 9\times10^{2}$	$2.0\times10^{3} \pm 3.5\times10^{3}$	$3\times10^{4} \pm 2\times10^{4}$	$6\times10^{2} \pm 8\times10^{2}$	$8\times10^{4} \pm 2\times10^{4}$
Nov.	$3.2\times10^{3} \pm 4.0\times10^{3}$	$2.3\times10^{2} \pm 1.7\times10^{2}$	$2.3\times10^{2} \pm 1.8\times10^{2}$	$2.0\times10^{4} \pm 1.8\times10^{4}$	$3.0\times10^{2} \pm 3.3\times10^{2}$	$4.5\times10^{3} \pm 6.7\times10^{3}$
Dec.	$4.5\times10^{3} \pm 9.7\times10^{3}$	$2.0\times10^{2} \pm 0.0$	$3.4\times10^{3} \pm 3.5\times10^{3}$	$2.5\times10^{4} \pm 2.7\times10^{4}$	$1.0\times10^{2} \pm 0.0$	$2.3\times10^{3} \pm 2.0\times10^{3}$
Jan.	$2.3\times10^{4} \pm 2.0\times10^{4}$	$1.2\times10^{3} \pm 1.3\times10^{3}$	$2.2\times10^{2} \pm 2.0\times10^{2}$	$1.1\times10^{5} \pm 1.8\times10^{5}$	$3.2\times10^{3} \pm 5.0\times10^{3}$	$5.0\times10^{2} \pm 4.3\times10^{2}$
Mar.	$1.4\times10^{4} \pm 1.3\times10^{4}$	$4.0\times10^{2} \pm 8.5\times10^{2}$	$1.0\times10^{3} \pm 1.7\times10^{3}$	$1.5\times10^{4} \pm 2.0\times10^{4}$	$1.5\times10^{2} \pm 71$	$1.0\times10^{2} \pm 0.0$
Apr.	$7.5\times10^{5} \pm 1.3\times10^{5}$	$2.0\times10^{3} \pm 3.6\times10^{3}$	$1.0\times10^{4} \pm 0.0$	$8.0\times10^{5} \pm 4.5\times10^{4}$	$3.0\times10^{3} \pm 4.5\times10^{3}$	ND

[a]Mean total mould count (CFU/g), $n = 80$

[b]Mean count of *Aspergillus* and *Penicillium* genera (CFU/g)

SD, standard deviation; ND, not detected

TABLE III
Mycoflora of rabbit feeds on DRBC and DG18 media

Month	DRBC (mould count ± SD)			DG18 (mould count ± SD)		
	Total[a]	*Aspergillus*[b]	*Penicillium*[b]	Total[a]	*Aspergillus*[b]	*Penicillium*[b]
May	$5 \times 10^2 \pm 5 \times 10^2$	ND	$5 \times 10^2 \pm 4 \times 10^2$	$4.0 \times 10^4 \pm 0.0$	$1.0 \times 10^2 \pm 0.0$	$1.0 \times 10^2 \pm 0.0$
July	$2 \times 10^3 \pm 2 \times 10^3$	$2 \times 10^3 \pm 1 \times 10^3$	$2.0 \times 10^3 \pm 1.5 \times 10^3$	$3 \times 10^2 \pm 2 \times 10^2$	$2 \times 10^2 \pm 8$	$1.0 \times 10^2 \pm 0.0$
Sept.	$3 \times 10^5 \pm 2 \times 10^5$	$1 \times 10^4 \pm 1 \times 10^4$	$9 \times 10^4 \pm 8 \times 10^4$	$6 \times 10^5 \pm 4 \times 10^5$	$1 \times 10^4 \pm 1 \times 10^4$	ND
Nov.	$2.0 \times 10^3 \pm 1.7 \times 10^3$	$4.0 \times 10^2 \pm 4.2 \times 10^2$	$2.0 \times 10^2 \pm 7.6$	$1.0 \times 10^4 \pm 6.7 \times 10^3$	$1.5 \times 10^2 \pm 0.0$	$2.0 \times 10^2 \pm 0.0$
Dec.	$2.4 \times 10^3 \pm 9.7 \times 10^2$	$1.0 \times 10^2 \pm 0.0$	$5.6 \times 10^3 \pm 4.3 \times 10^2$	$2.4 \times 10^3 \pm 6.8 \times 10^2$	$1.0 \times 10^2 \pm 0.0$	$4.4 \times 10^2 \pm 4.0 \times 10^2$
Jan.	$3.0 \times 10^3 \pm 4.4 \times 10^3$	$9.5 \times 10^2 \pm 1.2 \times 10^3$	$7.0 \times 10^3 \pm 0.0$	$9.3 \times 10^3 \pm 1.4 \times 10^4$	$1.0 \times 10^2 \pm 0.0$	$9.5 \times 10^3 \pm 0.0$
Mar.	$2.5 \times 10^2 \pm 50$	ND	$1.0 \times 10^2 \pm 0.0$	$3 \times 10^2 \pm 2 \times 10^2$	$1.0 \times 10^2 \pm 0.0$	$1.0 \times 10^2 \pm 0.0$
Apr.	$3.15 \times 10^4 \pm 3.50 \times 10^4$	$5.3 \times 10^2 \pm 4.6 \times 10^2$	$1.0 \times 10^2 \pm 0.0$	$5.0 \times 10^4 \pm 3.6 \times 10^4$	$3.8 \times 10^2 \pm 4.0 \times 10^2$	ND

[a]Mean total mould count (CFU/g), $n = 80$

[b]Mean count of *Aspergillus* and *Penicillium* genera (CFU/g)

SD, standard deviation; ND, not detected

TABLE IV
Count of *Aspergillus* section *Nigri* and *Circumdati* from poultry, pig and rabbit feeds in Argentina

Species	Count of species (CFU/g) ± SD[a]					
	Poultry		Pig		Rabbit	
	DRBC	DG18	DRBC	DG18	DRBC	DG18
A. niger var. *niger*	$1.35 \times 10^3 \pm 3.0 \times 10^3$	$3.6 \times 10^2 \pm 4.7 \times 10^2$	$3.4 \times 10^8 \pm 5.7 \times 10^8$	$1.0 \times 10^2 \pm 0.0$	$1.4 \times 10^8 \pm 6.0 \times 10^2$	$2.5 \times 10^2 \pm 1.8 \times 10^2$
A. niger var. *awamori*	$2.0 \times 10^2 \pm 2.0 \times 10^2$	$1.0 \times 10^2 \pm 0.0$	$1.0 \times 10^2 \pm 0.0$	$4.0 \times 10^8 \pm 6.3 \times 10^8$	$4.0 \times 10^2 \pm 5.2 \times 10^2$	$1.03 \times 10^3 \pm 9.5 \times 10^2$
A. foetidus	$2.0 \times 10^2 \pm 0.0$	$2.0 \times 10^2 \pm 0.0$	ND	ND	ND	ND
A. japonicus var. *japonicus*	$1.0 \times 10^3 \pm 0.0$	ND	$1.0 \times 10^2 \pm 0.0$	$1.0 \times 10^3 \pm 0.0$	$1.3 \times 10^3 \pm 0.0$	ND
A. japonicus var. *aculeatus*	$2.0 \times 10^2 \pm 0.0$	$1.0 \times 10^2 \pm 0.0$	ND	ND	ND	ND
A. ochraceus	$1.0 \times 10^2 \pm 0.0$	$1.0 \times 10^2 \pm 0.0$	ND	ND	ND	ND

[a]Mean values of count for each species (CFU/g), $n = 80$

SD, standard deviation; ND, not detected

compounds such as penitren A, xantomegnin, viomellein, vioxantin, rugulosins, rubratoxins and secalonic acid (Mills *et al*., 1995; Pitt and Hocking, 1997). These metabolites have been reported in cereal grains naturally contaminated with OA and citrinin (Scudamore *et al*., 1993; Scudamore and Patel, 2000). Some isolated species such as *P. glabrum*, *P. implicatum* and *P. pinophilum* have not been reported as mycotoxins producers. The *Aspergillus* and *Penicillium* species identified in this work agree with those reported by other authors in corn meal and different corn genotypes in Córdoba province of Argentina (Etcheverry *et al*., 1999). The fungal total counts during some months of sampling were higher than the maximum proposed limits, but in general the fungal total counts had moderate values between 10^2 and 10^4 CFU/g in all analysed feeds. It has been demonstrated that fungal propagules are helpful indicators for determining the hygienic quality of feeds. These counts should not exceed the 10^5 CFU/g (Chelkowski, 1991). Only very few countries have regulations for OA in food and feed products (Hohler, 1998). The levels of this toxin detected in feedstuffs were below regulatory limits (5–50 ng/g). These results showed the presence in feedstuffs not only of ochratoxigenic species of *Aspergillus* section *Nigri* but also of two strong OA producers, *P. verrucosum* and *A. ochraceus*. The presence of ochratoxigenic species and OA in feedstuffs indicates the risk of potential exposure of poultry, pigs and rabbits through the ingestion of feeds. The results for the occurrence of OA in feeds from different sampling years showed the dependence of OA production on conditions and storage time. Consequently, good storage practice becomes very important to prevent the growth of these fungi and OA production. As raw material is constantly arriving at the feed production plant from different storehouses, which cover a wide corn-producing region (south of Córdoba province), a correlation between ochratoxicogenic species or OA in feedstuffs and raw materials should be further investigated over the year.

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