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REVIEW

Occurrence of ochratoxin A and ochratoxigenic mycoflora in corn and corn based foods and feeds in some South American countries

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Abstract Cereals and cereal- derived products constitute the base of human and animal feeding in South American countries. This review attempts to give an overview of the ochratoxin A (OTA) occurrence and potential sources of OTA contamination in those products. The environmental conditions as humidity and temperature in the colonization of the substrates by *Aspergillus* section *Nigri* isolated from corn kernels were also discussed. The available information on the ochratoxigenic mycoflora and OTA presence in corn, corn based food and feed is limited. Only few surveys have been carried out in Argentina, Ecuador and Brazil; which showed that *Aspergillus niger* aggregate and *A. ochraceus* species would be the main source of OTA. It's possible to emphasize that, the species *A. carbonarius* has not been isolated from these substrates and *Penicillium verrucosum* was isolated only from pig feeds of Argentinean samples in low percentage. Studies about the ecophysiology of ochratoxigenic fungi and OTA occurrence are in progress in Latin America to reduce the impact of this toxin in the food chain.

Keywords *Aspergillus* section *Nigri* · *A. ochraceus* · Ochratoxin A · Corn- derived products

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Introduction

Ochratoxin A (OTA) is a mycotoxin that possesses a risk to human health due to its nephrotoxic, immunotoxic, mutagenic, teratogenic and carcinogenic biological properties. This toxin has been classified as a possible human carcinogen (category 2B) by the International Agency for Research on Cancer [1–3]. OTA was originally described as a metabolite of *Aspergillus ochraceus* grown in pure culture [4]. The oldest information indicates that *Penicillium verrucosum* is the main species associated to OTA production in foods and feeds in temperate climates, while *Aspergillus* spp. predominate in warmer and tropical countries. These species are

included in *Circumdati* and *Nigri* sections, such as *A. ochraceus*, *A. melleus*, *A. carbonarius* and *A. niger* aggregate [5–9].

Aspergillus section *Nigri* members (formerly *A. niger* group) are distributed worldwide, growing on a variety of substrates. They are considered as common food and feed spoilage fungi; *A. niger*, the most common species of section *Nigri*, is a recognized opportunist pathogen, its members are usually regarded as benign fungi. *A. niger* products hold the Generally Recognised As Safe status from the FDA (GRAS), despite the fact that OTA producing ability by this species has been reported [10, 11].

The natural presence of OTA in food and foodstuffs is widespread, especially in temperate climates. It is generally associated to a variety of products such as cereal, cereal based products, cocoa and coffee beans, grape juices, wine, dried fruit, spices, beer and products of animal origin [12–25].

The main contributors to OTA intake in humans and animals are cereals and cereal based food and feed. This fact occurs by the toxin resistance to technological processes [26]. Many countries have established regulatory limits for the presence of OTA in cereals and food based cereals [27]. In developed countries the maximum tolerable levels of OTA in cereals have been established in 5 ppb, but in food based cereals these levels are up to 3 ppb and in dried vine fruits up to 10 ppb. Whereas in other products such as food for infant and young children the limits are more strict, 0.5 ppb [28]. This paper reviews the available information on OTA contamination of corn and corn-based products, and details the dominant mycoflora found in South American data.

Morphological characteristics of *Aspergillus* section *Nigri*

Aspergillus carbonarius is the most distinct member of this section and the strains can be easily recognised using light microscopy. Their conidia are much larger than those of other black aspergilli and have echinulate conidial ornamentation. Conidia have more than 6 μm (mostly 7–9 μm) of diameter and are multinucleated [29, 30].

Various molecular analyses: Restriction Fragment Length Polymorphism (RFLP) and Random Amplification of Polymorphic DNA (RAPD) clearly differentiate *A. carbonarius* from the other biseriate black aspergilli [31, 32]. Considering the microscopic characteristics and molecular studies, *A. carbonarius* form a separate cluster inside the biseriate black aspergilli.

The taxa included in the so-called *Aspergillus niger* aggregate have been extremely difficult to distinguish one from the other by morphological means. When the strains grow on standard culture media and temperature regimes, it is possible to record certain characteristics for classification. These macroscopic (diameter, colour, texture, presence of furrows, pigments and exudates) and microscopic (stipes, vesiculae, metulae, fialides and conidia) characters allow to differentiate the species in the section *Nigri*. Members of the *A. niger* aggregate are characterized by their typically blackish colonies on all culture media used for classification [30]. Conidia are sometimes ornamented, globose to subglobose and with diameters of less than 6 μm. The *A. niger* aggregate was formed by two species, namely *A. foetidus* and *A. niger*, the latter was composed by six varieties and two formae. *A. foetidus* is differentiated by their colony diameter on Czapek agar, whereas the varieties of *A. niger* aggregate were basically identified by the roughness and ornamentation of their conidia. The authors differ in the time of incubation, 7 or 15 days up to 5 weeks for the observation of totally matured conidia [30, 33]. All the *A. niger* aggregate strains isolated from food and feed commodities develop blackish colonies and their morphological characteristics are difficult to differentiate; in some occasions are undifferentiated by classic taxonomy. In the last year, different molecular techniques have been applied to differentiate the species in the *A. niger* aggregate. RFLP, RAPD and karyotype analysis showed a high grade of variation among these strains. Through molecular studies *A. niger* aggregate have been grouped in to two main groups (I and II). These groups agree with the two species previously described, the strains of *A. niger* belonged to group I and the strains of *A. tubingensis* to group II [34, 35]. These studies

indicate that the strains belonging to the first group can be divided into various taxa. It is very difficult to distinguish them by morphological characteristics. Sometimes, the molecular techniques do not obtain a clear division between species and varieties belonging to *A. niger* aggregate [36, 37]. Accensi et al. [37], found a relationship between OTA-producing strains and morphological and molecular pattern. All the OTA-producing isolates whose known RFLP patterns were classified as type N, whereas none of the type T strains were able to produce this mycotoxin.

De Vries et al. [38], described a new species of the section *Nigri*, called *A. vadensis*, using morphological analysis, RFLP patterns and metabolite profiles. This species is closely related to *A. foetidus*, *A. tubingensis* and *A. niger* but differs on all examined characteristics.

Other species of this section such as *A. ellipticus* and *A. heteromorphus*, are not included in the last taxonomic key [30, 39, 40], because they are uncommon species in food and feeds. The extensive description of these species has been included in the first keys of *Aspergillus* genus [41].

Aspergillus japonicus and *A. aculeatus* are the only species in this section, which are uniseriates. Some authors distinguished these two taxa as separate species [33, 40, 41]. In Klich's manual [30] only *A. japonicus* is described, and *A. aculeatus* is mentioned only to point out the more important differences with *A. japonicus*. Other authors consider *A. aculeatus* as a variety of *A. japonicus*. [39]. The morphological descriptions are consistent and distinguish *A. aculeatus* (*A. japonicus var. aculeatus*) from *A. japonicus* (*A. japonicus var. japonicus*) mainly by its larger vesicles (more than 45 μm of diameter). The *Aspergillus* uniseriate strains develop compact, low and brown to black colonies on standard culture media used for their identification and isolation for natural substrates [42]. Their morphological characteristics are easily differentiated in comparison to the biseriate species. In molecular studies, some authors could distinguish both species by RFLPs of the mtDNA and rDNA. These investigations have shown that the uniseriate cluster was separated from the cluster of biseriate black Aspergilli [32, 43, 44]. Recently, the ITS-5.8S rDNA RFLP was applied in order to characterize the members of *Aspergillus niger* aggregate isolated from grapes [45, 46]. Another study, Perrone et al. [44] used the amplified fragment length polymorphisms (AFLPs) and genomic DNA sequences to identify black aspergilli isolated from grape berries from Italy. The 77 strains analyzed were clearly separated into four clusters: *A. tubingensis*, *Aspergillus uniseriate*, *A. carbonarius*, *and A. niger*, with a similarity of <20% for strains in different clusters.

Toxicogenic potential of *Aspergillus* section *Nigri*

Aspergillus niger isolates are known as producers of several toxic metabolites, such as formins and naphthopyrones among other secondary metabolites, but they have not been found as either cereal natural contaminant or toxic concern. *A. carbonarius* can also produce naphthopyrones and *A. aculeatus* produce secalonic acid D, this metabolite has significant animal toxicity but its role in animal disease has not been demonstrated and the studies of toxicity are scarce [40].

In the last years, the significance of black aspergilli has changed since the evidence that they can produce OTA. This mycotoxin is receiving increasing attention worldwide because of its toxicity to human and animal health.

Although some other *Aspergillus* spp. has been reported as ochratoxigenic [5]. It is worth noting that the main OTA-producing species are *Penicillium verrucosum* in temperate and cold climates, *A. ochraceus* and related species belonging to section *Circumdati* and *A. carbonarius* in warm and tropical climates.

The number of reports dealing with OTA production by members of section *Nigri* has been increasing since their first description carried out by Abarca et al. [47]. In this section *A. carbonarius* and *A. niger* are the confirmed OTA-producing species. The ability of the other species related to *A. niger* such as *A. awamori* and *A. japonicus* has been reported as OTA producers [6, 20, 48, 49] but this fact could be confirmed by molecular studies such as AFLP to confirm the classification of strains.

Mycoflora and potential OTA producing fungi in corn and corn based food and feed

Recently, Magnoli et al. [42] evaluated ochratoxigenic mycoflora from 50 samples of corn kernels for human consumption corresponding to 2003/2004-harvest season. These samples were obtained from a silo of a storing plant located in the south of Córdoba Province, Argentina. Among the *Aspergillus* section *Nigri*, the species *A. niger* var. *niger*, *A. niger* var. *awamori* and *A. japonicus* var. *japonicus* were isolated and identified. *Aspergillus flavus* was the most frequent mould, isolated in a 100% of the corn samples, followed by *A. niger* var. *niger* (56%) and *A. niger* var. *awamori* in 32 and 62% in dichloran rose bengal chloramphenicol medium (DRBC) and dichloran 18% glycerol medium (DG18), respectively (Figure 1). The percentage of corn kernels contaminated by *Aspergillus* species is summarized in Table 1. The percentage of corn kernels contaminated by *A. niger* var. *niger* was similar in both culture media, while the percentage of grains contaminated by *A. flavus* and *A. niger* var. *awamori* was higher than *A. niger* var. *niger* and *A. japonicus* var. *japonicus* ($P < 0.01$) in DG18 media. The other potentially ochratoxigenic species, *A. ochraceus*, was isolated between 5 and 10% of the corn kernels in DG18 and DRBC media, respectively. Table 2 shows the potential for OTA production by *Aspergillus* spp. isolated from corn kernels. Among 112 black *Aspergillus* strains, thirty (25%) were OTA producers on YES (2% yeast extract, 15% sucrose) medium, with levels ranging from 2 to 31.5 ng ml^{-1}. A high percentage (36%) of strains with morphological characteristics corresponding to *A. niger* var. *awamori* were OTA producers; the mean levels of toxin ranged from 2.5 to 21.5 ng ml^{-1}; while 17% of *A. niger* var. *niger* were OTA producers, with mean levels ranging from 3.0 to 31.5 ng ml^{-1}. Only one of four strains of *A. ochraceus* was OTA producer.

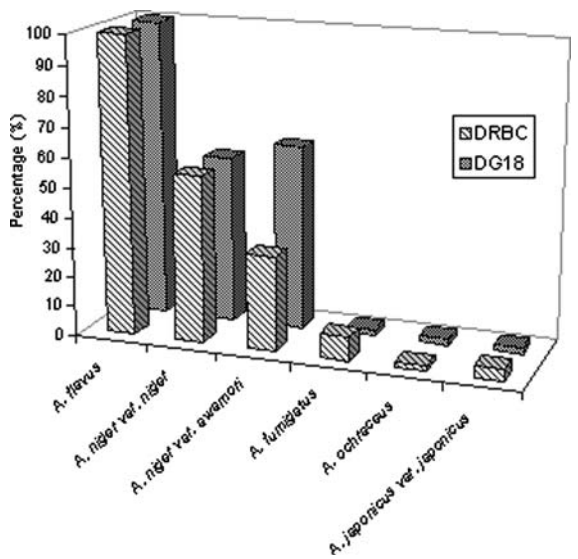

Fig. 1 Isolation of *Aspegillus* species in com samples from Argentina [42]

The mycoflora and the potential for OTA production were evaluated by Magnoli et al. [50], from corn-based feeds (poultry, pig and rabbit feeds). A total of 80 samples were taken at random from factories located from Córdoba province, Argentina, over a period of eight months. 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 in a higher frequency from rabbit feeds. The distribution of section *Nigri* species, varied according to the feedstuffs analysed. In general, the percentage of potentially ochratoxigenic species showed differences regarding the culture media in which the isolation was done. From poultry feeds, *A. niger* was isolated in frequencies of 12.5% and 27.5% on DG18 and DRBC media, respectively (Figure 2). In pig and rabbit feeds, this species was present in 7.5 and 10%, respectively (Figure 3 and 4). *A. awamori* strains, was isolated from 2.5 to 17.5% of poultry, pig and rabbit feeds, and *A. japonicus* from 2.5 to 5% of the three feed samples. Other species of this section, *A. foetidus* and *A. aculeatus* (2.5%), were isolated only from poultry feeds. From the *Circumdati* section, *A. ochraceus* was present in low number of samples (2.5%) from poultry feeds (Figure 2). Likewise, the potentially ochratoxicogenic species *P. verrucosum* was isolated in low frequency (2.5%) from pig feeds (Figure 3) and *A. carbonarius* species was not isolated from these substrates. *Aspergillus* section *Nigri* species occurred in moderate mean

Table 1 Percentage of corn kernels contaminated by *Aspergillus* species from Argentina [42]

Species	Percentage of corn kernels contaminated			
	DG18		DRBC	
	Range (%)	Mean (%)*	Range (%)	Mean (%)*
A. flavus	12–54	33[a]	6–56	21[b]
A. ochraceus	4–6	5	10	10
A. fumigatus	6	6	2–10	6
A. niger var. *niger*	2–8	4[c]	2–8	4.3[c]
A. niger var. *awamori*	2–14	6[c]	2–12	4.4[c]
A. japonicus var. *japonicus*	2	2[c]	2–4	3[c]

* Mean percentage of corn kernels contaminated

$n = 50$

[a, b, c] Letters in common are not significantly different according to Fisher's protected LSD test ($P < 0.01$)

Table 2 Ochratoxin A production by *Aspergillus* section *Nigri* species isolated from Argentinean corn kernels [42]

Species	Positive strains[a]	Range of OTA (ng ml^{-1})[b]	Mean levels (ng ml^{-1}) ± SD
A. niger var. *niger*	4/57	2.0–4.0	3.0 ± 0.8
	5/57	5.0–6.0	5.5 ± 0.5
	1/57	31.5	31.5 ± 0.0
A. niger var. *awamori*	11/55	2.0–3.0	2.5 ± 0.4
	5/55	4.0–8.0	6.0 ± 1.7
	3/55	8.5–11	9.75 ± 2.0
	1/55	21.5	21.5 ± 0.0
A. ochraceus	1/4	8.0	8.0 ± 0.0

[a] Number of OTA producers strains vs. total strains

[b] OTA was detected by HPLC method. Detection limits: 1 ng ml^{-1}

SD: standard deviation

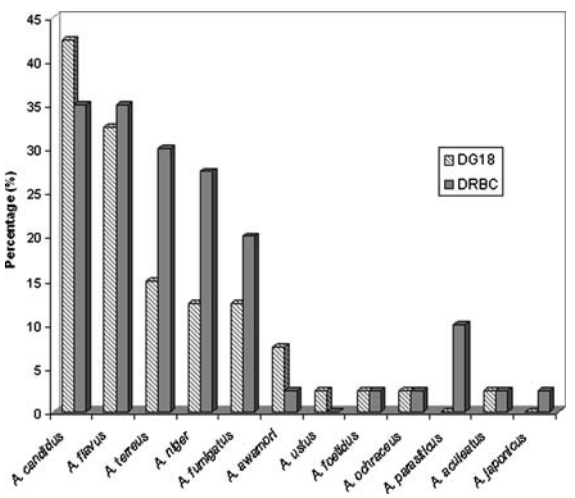

Fig. 2 Percentage of *Aspergillus* species in poultry feed samples from Argentina [50]

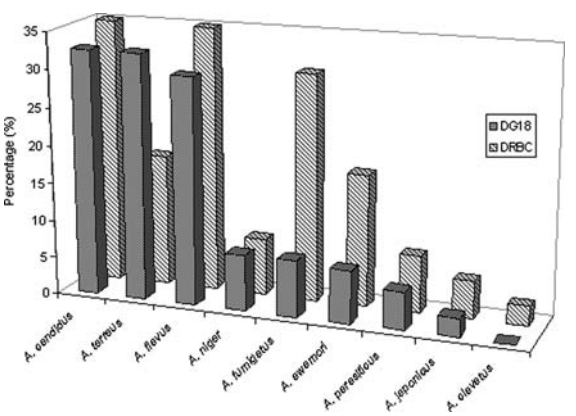

Fig. 3 Percentage of *Aspergillus* species in pig feed samples from Argentina [50]

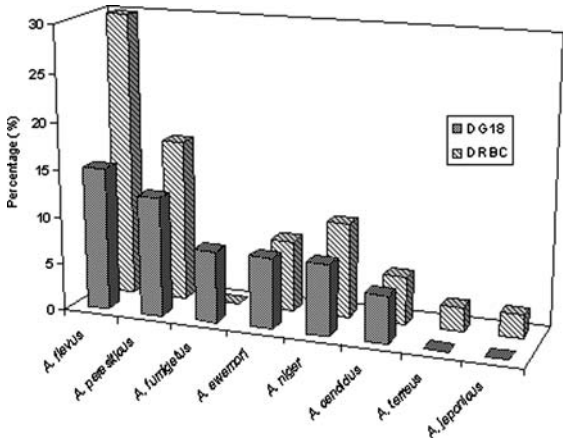

Fig. 4 Percentage of *Aspergillus* species in rabbit feed samples from Argentina [50]

colony counts from three feeds, the colony count analysis was summarized in Table 3.

In another study from Argentina, black *Aspergillus* strains isolated from poultry, pig and rabbit feeds were tested for OTA production in YES medium. The highest percentage of ochratoxigenic strains was isolated from rabbit feeds with 100% of *A. niger* producing strains; 78% of strains with morphological characteristics corresponding to *A. awamori* were OTA producers, with mean levels of 15.0 and 16.5 ng ml^{-1}, respectively. From pig feeds, 61% of *A. awamori* were producers of this toxin with mean levels of 16 ng ml^{-1}. In poultry feeds, the lowest percentage of OTA producer strains was detected (Table 4) [20].

Recently, in Rio de Janeiro State, Brazil, a survey was carried out to evaluate the mycoflora and the ability to produce OTA by *Aspergillus* spp. from 144 samples of maize, initial poultry feeds, pelleting poultry feed and trough feed samples. Total maize mycobiota counts ranged between 1×10^4 and 7×10^4 CFU g^{-1} and total initial poultry feed mycobiota counts varied between 4×10^3 and 3×10^4 CFU g^{-1}. When the pelleting process was done these counts decrease significantly; nevertheless they were below the limit of detection (100 g^{-1}). In maize and initial poultry feed samples *A. flavus* and *Eurotium chevalieri* were the more prevalent species isolated (28%), followed by *E. amstelodami*, *A. candidus* and *A niger*. In trough feed samples,

Table 3 Count of *Aspergillus* section *Nigri* and *Circumdati* from poultry, pig and rabbit feeds in Argentina

Species	Count of species CFU $g^{-1} \pm SD^a$					
	Poultry		Pig		Rabbit	
	DRBC	DG18	DRBC	DG18	DRBC	DG18
A. 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. 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	$1.0 \times 10^3 \pm 0.0$	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. 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^{-1})

SD: standard deviation

nd: no detected

n: 80

Table 4 Ochratoxin A production by *Aspergillus* section *Nigri* species isolated from Argentinean feedstuffs [20]

Substrate	Species	Positive strains[a]	Range of OTA (ng ml^{-1})	Mean levels (ng ml^{-1})[b] ± SD
Poultry feed	*A. niger*	5/17	13.3–16.3	14.3 ± 3.0
	A. awamori	2/15	13.2–15.5	14.35 ± 2.0
Pig feed	*A. niger*	2/6	14.2–14.8	14.5 ± 3.6
	A. awamori	11/18	13.2–23.6	16 ± 2.5
	A. japonicus	2/6	13.3–14.0	13.65 ± 2.0
Rabbit feed	*A. niger*	14/14	13.3–24.7	15.05 ± 2.6
	A. awamori	7/9	13.0–16.0	16.5 ± 2.6

[a] Number of OTA producer strains vs total strains

[b] OTA was detected by HPLC method. Detection limit: 1 ng ml^{-1}

SD: standard deviation

A. flavus, *E. amstelodami* and *E. chevalieri* were the more frequent species, isolated from 22% of the samples, followed by *A. niger*, *A. sydowii* and *A. versicolor*. Two of four *A. melleus* strains were OTA producers on YES agar medium, at level of 0.344 μg ml^{-1} (data not shown). The variability of the species found in the different types of samples could be attributed to the different composition of foods assayed, Fraga et al. [51].

Rosa et al. (2006) analysed 96 Brazilian poultry feed samples. *A. flavus* was the most prevalent (50% of the samples) followed by *A. niger*, *A. ochraceus* and *A. melleus* in a 14, 12 and 8% of the samples, respectively. Table 5 shows the ochratoxigenics capacity of *Aspergillus* and *Penicillium* species isolated from these samples.

Pozzi et al. [52], evaluated the toxigenic mycoflora from 130 samples of corn, at post harvest and stored stage, originated from Sao Paulo, Brazil. This region is characterized by humid tropical weather. *Aspergillus* spp. was isolated from corn post harvest and stored at 40.7% of the samples. Only two ochratoxigenic species: *A. niger* (11.5%) and *A. alutaceus* (6.1%) were found.

In data from Ecuador, the natural mycoflora was analysed in samples of corn from farms at harvest (coastal and mountain regions) and samples of corn- based pellets feed. The fungi associated with corn-based pellets destined for poultry, pig and cow feeds in the Ecuadorian mountain region showed that *A. flavus* was the most prevalent fungus present followed by *Fusarium graminearum*, *F. verticillioides* and *A. parasiticus*. In this substrate, *A. niger* was not isolated. The predominant *Aspergillus* spp. isolated from corn, was *A. flavus* followed by *A. niger* and *A. parasiticus* isolated at 60, 20 and 10% of the samples from the coastal region, respectively. Whereas, in the mountain region, these species were isolated at 25, 12.5 and 4.2% of the samples, respectively. Significant differences in the isolation relative density of *A. flavus* and *A. parasiticus* were observed between the two regions, but there was no significant difference for *A. niger* [53].

Table 5 Ochratoxin A production by *Aspergillus* and *Penicillium* strains isolated from Brazilian poultry feed samples on CYA medium [54]

Species	Positive strains[a]	Range of OTA (μg kg^{-1})[b]	Mean levels (μg kg^{-1}) ± SD
A. niger	43/175	10–26	17.2 ± 5.3
A. carbonarius	5/7	9–32	22.6 ± 7.3
A. ochraceus	19/74	53–116	96.5 ± 18.6
A. melleus	9/23	36–67	44.7 ± 13.2
P. verrucosum	8/61	18–97	52.8 ± 11.7

[a] Number of OTA producer strains vs total strains

[b] OTA was detected by HPLC method. Detection limit: 0.4 ng g^{-1}

SD: standard deviation

The mycoflora and OTA production was evaluated by Rosa et al. (Personal Communication) from 109 samples of raw materials including corn, brewer's grain and barley rootlets used as ingredients and finished cow feed samples. These samples were collected in Riode Janeiro State, Brazil. The mycological survey of corn samples showed that *Aspergillus* spp. and *Fusarium* spp. were isolated from 60% of the samples, and *Penicillium* spp. in lower frequency (40%). These genera were prevalent in other cereals, the frequency of isolation varied according to the substrate. *A. flavus* was isolated from corn, brewer's grain and finished cow feed in 34, 14 and 32% of the samples, respectively. *A. carbonarius* specie, was only isolated from brewer's grain in low percentage of samples. *A. niger* and *A. ochraceus*, were found in all substrates in 20–40% and 10–25% of the samples, respectively. Whereas, *P. verrucosum* specie was isolated from corn, brewer's grain and barley rootlets in 8, 15 and 17% of the samples, respectively. The evaluation of the ability to produce OTA on YES culture medium showed that 71% of *A. carbonarius* strains were OTA producers, 38% of *A. niger*, 33% of *A. ochraceus* and 13% of *P. verrucosum*. OTA levels ranged from 16 to 116 μg kg^{-1}. *A. ochraceus* and *P. verrucosum* strains produced the highest OTA levels followed by *A. carbonarius* and *A. niger*. High percentages of potential OTA producer strains were found. They represent 40.6, 69, 46 and 38% on corn, brewer's grain, barley rootlets and finished cow feed, respectively.

Natural occurrence of OTA in corn and corn based food and feed

Only one evaluation of the natural incidence of OTA from corn kernel samples destined for human consumption corresponding to 2003/2004- harvest season was carried out in Córdoba province, Argentina. These samples were analysed at storage. All corn samples were OTA negative at the detection limit of the technical applied of 1 ng g^{-1} [42].

In a previous work the natural incidence of OTA in corn based feeds: poultry, pig and rabbit feeds from Argentina were analysed. This toxin was found in 38% of the poultry feed samples with levels ranging from 25 to 30 ng g^{-1}. From rabbit feed samples, 25% contained OTA, and the levels ranged from 18.5 and 25.5 ng g^{-1}. Only 13% of pig feed samples was contaminated with similar levels of toxins (Table 6) [20]. In a later study with these substrates OTA was detected in 10%, 15% and 12% of poultry, pig and rabbit feed samples, respectively. The mean levels detected ranged from 15 to 25 ng g^{-1} from three feeds [50].

Table 6 Incidence of OTA from Argentinean feedstuffs[20]

Month	Ochratoxin A					
	Poultry		Pig		Rabbit	
	(%) Positive sample[a]	Levels (ng g^{-1})[b] ± SD	(%) Positive samples [a]	Levels (ng g^{-1})[b] ± SD	(%) Positive samples [a]	Levels (ng g^{-1})[b] ± SD
May	0	n.d.	0	nd	0	nd
July	0	n.d.	0	nd	0	nd
September	0	n.d.	0	nd	0	nd
November	100	26 ± 0.4	100	34 ± 1.04	0	nd
December	100	30 ± 0.06	0	nd	100	18.5 ± 0.03
January	100	25 ± 0.1	0	nd	100	25.5 ± 0.4
March	0	n.d.	0	nd	0	nd
April	0	n.d.	0	nd	0	nd

[a] Percentage of positive samples from five samples for each month

[b] Mean of concentration OTA. Toxin was detected by HPLC method. Detection limit: 10 ng g^{-1}

SD: standard deviation

nd: no detected

In Rio de Janeiro, Brazil, Fraga et al. [51] evaluated the natural occurrence of OTA from 144 samples of maize, initial poultry feeds, pelleting poultry feed and trough feed samples. Final poultry feed samples showed that 100% were contaminated with OTA with levels from 17 to 197 μg kg^{-1} (mean value: 98.2 μg kg^{-1} ± SD: 22.3). These authors concluded that although the pelleting process produced an important fungal reduction, this did not occur with mycotoxins. These results agree with Rosa et al. [54], from the same investigative centre, who obtained the same percentage of OTA contaminated samples at lower levels, which varied from 1.3 to 80 μg kg^{-1}.

Caldas et al. [55], analysed from July 1998 to December 2001 the presence of aflatoxins and OTA in several food samples from Brazil including maize and maize based products (popcorn and kernels). Samples were processed and the extracted mycotoxins were detected and separated using thin layer chromatography, and thin quantified with fluorescence. OTA was not detected (<25 mg kg^{-1}) in any sample analysed.

A total of 109 samples of raw materials including corn, brewer's grain and barley rootlets used as ingredients and finished cow feed samples were collected from Riode Janeiro State, Brazil. The samples were analysed for OTA contamination by HPLC using immunoaffinity columns. This study showed that brewer's grain samples were the most contaminated (45%), showing OTA levels between 27 and 439 μg kg^{-1}. Thirty-one percent of corn and 22% of barley rootlets were contaminated with OTA at levels ranging from 4.9 to 132 μg kg^{-1} and 20 to 637 μg kg^{-1}, respectively. Twenty-five percent samples of finished cow's feed samples were positive for OTA with levels ranging from 12 to 324 μg kg^{-1} (Rosa et al., Personal Communication).

Environmental factors affecting OTA production

In black aspergilli species, the ochratoxin A production on cereals and derivates is the consequence of the interaction of different factors such as fungal species, composition of the substrate and environmental including oxygen, temperature, humidity, carbon dioxide, pH and incubation time. The environmental conditions as humidity and temperature have a strong influence in the colonization of the substrates and in the amount of OTA produced by the different strains [56].

The knowledge of the optimal and marginal temperatures and the levels of water activity (a_W) for fungal proliferation and the toxin formation can be useful to optimize the conditions of pre and post-harvest as well as in storage. Esteban et al. [57] have demonstrated that similar to other OTA producer species, temperature is one of the most important factors which influences *A. niger* aggregate and *A. carbonarius* strains for OTA production.

It is known that optimum conditions of a_W and temperature for mycotoxin production are more restrictive than those for fungal growth [58]. Nowadays, several authors have investigated the influence of water activity, temperature and incubation time on growth and OTA production by *Aspergillus* section *Nigri* strains isolated from grapes and derived products in Europe countries [59–63]. However, very little is known about the optimal conditions for growth and OTA biosynthesis by *A. niger* aggregate and *A. carbonarius* in South America. In a recent work, Astoreca et al. [64], determined the effect of a_W, temperature, and their interactions on mycelial growth rate and the lag phase prior to growth of *A. awamori* strains isolated from Argentinean corn kernels. These strains showed that the optimal a_W level for growth was 0.97 with optimal temperature of 30°C.

In another study, carried out with the same strains their capacity to produce OTA at different environmental conditions on corn kernels meal extract agar was investigated. In the assayed strains, the optimum conditions for OTA production were found at the highest a_W (0.99) after 7 days of incubation at 25°C (Astoreca et al., personal communication). These studies were carried out with other species of *Aspergillus* section *Nigri* isolated from other substrates (dried grapes, peanuts and coffee cherries) (Astoreca et al. personal communication).

Current scientific literature on the influence of environmental factors on OTA production has been focused mainly on *A. ochraceus* and

P. verrucosum strains isolated from cereals from European countries [65–67]. Few studies have reported the effects of environmental conditions such as water activity, temperature and incubation time on the levels of OTA produced by *Aspergillus* spp. isolated from South America countries [68, 69].

Conclusion

Aspergillus carbonarius, *A. niger* aggregate species and *A. ochraceus* are the main OTA producers in South American countries. Water activity, is one of the main factors, highly influence OTA contamination of several substrates because it favours overgrowth of OTA-producing black aspergilli. Significant amounts of OTA can be produced in only 7 days at $a_W \geq 0.97$ at a wide range of temperature on corn kernels meal extract agar. This fact indicates that potential dangers of OTA contamination occur if the storage conditions of these substrates are unfavourable. The knowledge of the ecophysiology of *Aspergillus* section *Nigri* is critical in the development and prediction of the risk models of contamination of raw materials and final products by these species under fluctuating and interacting environmental parameters.

It is generally assumed that the mycotoxin problem is more serious in developing countries where the climatic conditions and the agricultural and storage practices are considered conductive to fungal growth and toxin production. Furthermore, South American countries do not have enough economical sustenance to control the storage conditions and to prevent, also, the fungal contamination in cereals and based cereal derived. Most of these countries lack of National Programs for the control of OTA in food; consequently there are scarce data to implement regulations to protect population's health. Considering that corn is one of the most important cereals in Argentina, not only in production but also in export, OTA prevention on this substrate becomes highly important.

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