FUNGAL AND MICOTOXIN CONTAMINATION IN MIXED FEEDS: EVALUATING RISK IN CATTLE INTENSIVE REARING OPERATIONS (FEEDLOTS)

CONTAMINACIÓN CON HONGOS Y MICOTOXINAS EN RACIONES DE BOVINOS: EVALUACIÓN DE RIESGO EN ESTABLECIMIENTOS DE CRÍA INTENSIVA (FEEDLOTS)

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Abstract

Argentina is the fourth global beef producer. Exposure to mycotoxins through contaminated feed is a major hazard for ruminants. In the present study we assess mycobiota, aflatoxin B_1 (AFB_1), fumonisin B_1 (FB_1), deoxynivalenol (DON) and zearalenone (ZEA) levels in total mixed rations (TMRs) during two consecutive years. Total fungal counts were evaluated and fungal species were identified. Also, ability of *A. flavus* isolates to produce AFB_1 *in vitro* was tested. Natural contamination with AFB_1 and FB_1 was quantified by HPLC. Deoxynivalenol and zearalenone were analysed by immunochromatography and thin-layer chromatography (TLC), respectively. Fungal counts varied from not detectable (ND) to 2.10×10^8 CFU g^{-1}. The prevalent genera were *Aspergillus* spp (60 %) and *Fusarium* spp (66.7 %), respectively. The prevalent species was *Aspergillus fumigatus*. 50 % of *A. flavus* strains produced 75 to 112.5 µg g^{-1} AFB_1. 46 % of 2007 samples were contaminated with 4 to 10 µg kg^{-1} AFB_1. Deoxynivalenol was detected in 33.3 % of the samples (≥ 1. 25 µg g^{-1}). Fumonisin B_1 and ZEA were not detected. This study can be useful to estimate the mycotoxicological risk of cattle TMRs in this region and to compare results with studies from other beef-producing countries.

Key words: Aflatoxins; cattle; deoxynivalenol; feedstuffs; fumonisins.

Resumen

Argentina es el cuarto productor mundial de carne. Por ello, la exposición a

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micotoxinas a través de alimentos contaminados representa un gran riesgo para el ganado. En el presente trabajo, se evaluó la micobiota y contaminación natural con aflatoxina B_1 (AFB_1), fumonisina B_1 (FB_1), deoxinivalenol (DON) y zearalenona (ZEA) en raciones completas mixtas (TMRs) para bovinos, durante dos años consecutivos. Para ello, se evaluaron los recuentos fúngicos totales (UFC g^{-1}), se identificaron las especies, se evaluó la capacidad toxicogénica de las cepas de *A. flavus in vitro* y se cuantificaron los niveles de micotoxinas por HPLC, inmunocromatografía y cromatografía en capa fina (TLC). Los recuentos variaron entre no detectable (ND) y 2.10 x 10^8 UFC g^{-1}. Los géneros aislados con mayor frecuencia fueron *Aspergillus* spp (60 %) y *Fusarium* spp (66.7 %), mientras que La especie prevalente fue *A. fumigatus.* El 50 % de las cepas de *A. flavus* aisladas produjeron entre 75 y 112.5 µg g^{-1} de AFB_1. El 46 % de las muestras recolectadas en 2007 presentaron contaminación con 4 a 10 µg kg^{-1} de AFB_1. En el 33 % de las muestras se encontraron niveles de DON ≥ 1.25 µg g^{-1}. No se encontraron niveles detectables de FB_1 ni ZEA. Este estudio es útil para estimar el riesgo micológico de las raciones usadas para la cría intensiva de bovinos en una de las principales regiones productoras de bovinos de carne y comparar los resultados con estudios realizados en otros países.

Palabras clave: Aflatoxinas; deoxinivalenol; fumonisina; ganado; raciones.

Introduction

Argentina is the fourth most important beef producer in the world. Bovine meat is consumed daily in the basic diet of its population (56.5 kg/year/person) and constitutes one of the main products for export. Argentine beef quality was known worldwide mainly because cattle were 100 % grass-fed. However, in recent years, feedlot rearing has increased due to the use of great extensions of land for agriculture, particularly soybean cultivation.

Mycotoxins are low molecular weight products of the fungal secondary metabolism, produced mainly by A*spergillus, Penicillium, Fusarium* and *Alternaria* genera. They are toxic to humans and animals when consumed or inhaled. Exposure to mycotoxins through contaminated feed is one of the major risk factors to ruminant health (Kalac & Woolford, 1982; Bennett & Klich, 2003). Aflatoxins (AFs) are potent carcinogenic toxins and ingestion of hepatotoxic AFB_1 can induce the presence of aflatoxin M_1 in milk (Corbett *et al*., 1988). Acute aflatoxicosis in cattle has been thoroughly described. Consumption of feed containing high levels AFs may reduce growth rate and increase the amount of feed required per pound of gain. High levels of AFs can cause liver damage in adult cattle and depress immune function, resulting in disease outbreaks (CAST, 2003). Fumonisins (FBs) have been associated to the occurrence of pulmonary edema in pigs, leukoencephalomamacia in horses, hepatic cancer in rats and esophageal cancer in humans (Howard *et al.,* 2001; Marasas, 2001; Smith *et al*., 2002). However, cattle appear to be quite resistant to FBs due to limited absorption and metabolism (Rice & Ross, 1994). Other mycotoxins such as trichothecenes and zearalenone (ZEA) alter immune-mediated activities in bovines (Black *et al*., 1992). Occurrence of mycotoxins such as AFs, FBs, ZEA, T_2 toxin and gliotoxin in cattle feeds and ingredients has been already reported (Batatinha *et al*., 2007; Naicker *et al*., 2007; Simas *et al*., 2007; Lanier *et al*., 2009; Lanier *et al*., 2010). Simas *et al*., (2007) encountered *Aspergillus* spp*, Penicillium* spp and *Fusarium* spp*,* in brewer's grains samples intended for cattle.

Total mixed rations for cattle (which are prepared in the farm immediately prior to feeding) are based on corn grains and added a commercial protein concentrate, a dietary fiber source and a vitamins/minerals pre-blend. Corn or sorghum silage made out of entire plants is often added to the diet to accelerate weight gain. Great volumes of the different ingredients that compose TMRs are stored in the farms, often in

not sufficiently clean reservoirs and for long periods of time, increasing the risk of mould and mycotoxins contamination from air, soil and the same stored material. Surveillance for mycotoxins in cereals and animal feeds has reported that more than one toxin can occur in the same commodity (Scudamore & Livesey 1998). Most studies describe fungal and mycotoxin contamination in different feed ingredients (Dutton & Kinsey, 1995; Salay & Zerlotti Mercadante, 2002; Richard *et al*., 2007; Simas *et al*., 2007). However, there is little information concerning the level of contamination of the TMRs just as they are consumed by the animals.

If toxigenic fungi contaminate TMRs or their ingredients, these fungi and their mycotoxins should be isolated from the feed in the bunks. The aims of this study were i) to evaluate the mycobiota and ii) to quantify levels of mycotoxins – AFB_1, FB_1, deoxynivalenol (DON) and ZEA – in finished feed intended for intensive rearing beef cattle during two consecutive years.

Materials and methods

Sample collection

Forty samples of TMRs were collected from different feedlots from May to November 2006 and from May to November 2007. The farms were located in the province of San Luis, in the semi-arid region of Argentina in which, as well as in the pampas, bovine rearing is a central activity. They included medium to large stockyards, confining 25,000 to 40,000 animals. Temperature in this region ranged between -5 and 39 °C with an annual average of 17 °C. The annual rain average varied between 300 and 700 mm. Two model diets are described in Table 1.

Two kg samples were collected at random, comprising material from different bunks. As cattle feed is mixed during transport in the delivery vehicle (mixer), collecting the sample from the feed bunk is the most appropriate. Samples were homogenized and quartered to get 500 g laboratory samples. A 10 g aliquot from each sample was randomly selected for the analysis of the mycobiota and the remaining sample was dried at 65 °C, milled and stored at 4 ºC until mycotoxin analyses.

Analysis of the mycobiota

Total fungal counts were performed on two different culture media: dichloran rose bengal chloranphenicol agar (DRBC), for estimating total mycobiota, and dichloran 18 % glycerol agar (DG18), a low a_W medium, to isolate xerophilic fungi (Pitt & Hocking, 1997). Quantitative enumeration was done using the plate count method as described in ISO 21527-1 and

Table 1.
Composition of TMRs used in two different feedlots

Cattle feed composition (%)			
Diet 1		Diet 2	
Corn silage	20	Alfalfa hay	15
Corn grain	60	Corn grain	70
Gluten feed	10	Gluten feed	10
Peanut shells	5	Wheat bran	Variable amounts[a]
Concentrate (monensine, urea and minerals)	1.5	Concentrate (monensine, urea and minerals)	5
Soybean or wheat pellet	3.5		

[a]Adequate amounts were added to the diet in order to reach 10 % acid detergent fiber (ADF) content.

ISO 21527-2. 10 g of each sample were homogenized in 90 mL 0.1 % peptone water solution for 30 min in an orbital shaker. Serial dilutions (10^{-2} to 10^{-3}) were made and 0.1 mL aliquots were inoculated in duplicates on the culture media. Plates were incubated at 25 ºC for 7-10 days in the dark. Only plates containing 15-150 colony-forming units (CFU) were used for counting. The results were expressed as CFU per gram of sample (CFU g^{-1}). Representative colonies of *Aspergillus* and *Penicillium* spp. were sub-cultured in tubes containing malt extract agar (MEA) and *Fusarium* spp. were transferred to carnation leaf agar (CLA) plates. Fungal species were morphologically identified according to Nelson *et al*. (1983), Samson *et al*. (2000), Klich (2002) and Frisvad and Samson (2004). The results were expressed as: i) isolation frequency of the fungal genera (defined as the percentage of samples in which each genus was present in relation to the total number of samples) and ii) relative density of each fungal species (defined as the percentage of each species among the total number of isolates of a certain genus).

Mycotoxigenic capacity

Ability of potentially toxigenic *Aspergillus* section *Flavi* isolates to produce AFB_1, AFB_2, AFG_1 and AFG_2 in MEA medium *in vitro* was tested according to methodology described by Geisen *et al*. (1996). Five day cultures growing in MEA at 30 °C were extracted with 500 µL chloroform and centrifuged 20 min at 3000 rpm. The mycelia was removed, the extracts were evaporated under N_2 flow and redissolved in 200 µL chloroform. 2, 5 and 10 µL drops were spotted on silica gel 60 TLC aluminum sheets (20 x 20 cm, Merck™, Germany) along with 2, 5 and 10 µL spots of a quantified aflatoxin standard solution (containing AFB_1, AFB_2, AFG_1 and AFG_2). Mobile phase was chloroform:acetone (90:10 v/v). The chromatograms were observed under 365 nm UV light and aflatoxin content of extracts was quantified by comparing intensity of fluorescence of the spots with the standard solutions.

Mycotoxin Analyses

The natural incidence of four mycotoxins in cattle TMRs was evaluated. The presence of AFB_1 was evaluated by HPLC. Extraction was carried out using AflaPat Mycosep®228 clean-up columns (Romer Labs Inc., Union, MO, USA) following the directions supplied by the manufacturer. Each extract was evaporated to dryness under N_2 flow and redissolved in 400 µL mobile phase acetonitrile:methanol:water (17:17:66 v/v). An aliquot (200 µL) was derivatized with 700 µL trifluoroacetic acid:acetic acid:water (20:10:70 *v/v*) and analyzed by HPLC according to methodology described by Trucksess *et al.* (1994). Quantification limit of the method was 5 ng g^{-1}. Fumonisin B_1 content of TMRs was determined as follows: 25 g of feed were ground with a grinder (20 mesh; 840 µM particle size) and extracted with 100 mL of methanol: water (3:1, *v/v*). The mixture was shaken for 30 min with an orbital shaker and filtered through Whatman Nº 4 paper 4 (Whatman, Inc., Clifton, New Jersey, USA). The extracts were analysed for FB_1 by HPLC using the method proposed by Shephard *et al.* (1990) and modified by Doko *et al.* (1995). For HPLC analysis of FB_1 a 50 µL aliquot of each extract was derivatized with 200 µL *o*-phthaldialdehyde (OPA) solution. The OPA solution was obtained by adding 5 mL of 0.1 M sodium tetraborate and 50 µL 2-mercaptoethanol to 1 mL of methanol containing 40 mg of OPA. 20 µL of the derivatized extracts were injected into the HPLC. The HPLC system consisted on a Hewlett Packard 1050 pump (Palo Alto, CA, USA) connected to a Hewlett Packard 3395 integrator. The column used was a C18 RP Phenomenex Luna (150 x 4.60 mm, 5 µ) (Phenomenex, USA). The mobile phase was a methanol:0.1 M dihidrogenated sodium phosphate (75:25) solution, pH 3. 35. Flow was fixed to 1.5 mL min^{-1}. Fumonisin B_1 was quantified by comparing peak height measurements with a reference standard solution. The standard solution was obtained by dissolving crystalline FB_1 (Division of Food Science and Technology, Preto-

ria, South Africa) in acetonitrile:water (1:1). A five point standard curve was developed to quantify FB_1. Quantification limit of the method was 20 ng g^{-1}. For DON semiquantitative analyses, commercial RIDA®QUICK DON Immunochromatographic Test kits were used (R-Biopharm AG, Darmstadt, Germany) following the protocol provided by the manufacturer. The detection limit of the method was 0.5 µg g^{-1}. Zearalenone analysis was performed by TLC as it is described in the Official Methods of Analysis (AOAC, 1995). The extracts were screened for ZEA contamination by spotting 2, 5, and 10 µL of each along with ZEA standard solutions on a silica gel 60 TLC aluminum sheet (20 x 20 cm, Merck™, Germany) and developed with chloroform:acetone (90:10 v/v). Chromatograms were air dried and observed under 254 nm UV light. The relative amount of ZEA was quantitatively determined by visual comparison under UV light with standard solutions of known toxin concentration. Detection limit was 5 µg g^{-1}.

Statistical analyses. Data analyses were performed by analysis of variance (ANOVA). Fungal counts data were transformed using the logarithmic function $\log_{10}(x + 1)$ before applying ANOVA. Duncan's test was used for comparing CFU g^{-1} total fungal counts on different culture media and Fisher's protected LSD test was used for comparing means of mycotoxins contamination data (Quinn & Keough, 2002). The analyses were conducted using PROC GLM in SAS (SAS Institute, Cary, NC, USA).

Results and Discussion

Analysis of the mycobiota

Total fungal counts present in cattle TMRs varied from less than 1 x 10^3 CFU g^{-1} (detection limit of the method) to 2.10 x 10^8 CFU g^{-1} in DRBC and from ND to 1.8 x 10^8 CFU g^{-1} in DG18. When comparing both DRBC and DG18 counts of the different sampling periods, higher counts could be observed during 2007. 90 % of counts from 2006 were greater than 107 CFU g^{-1} in DRBC and 10^6 CFU g^{-1} in DG18 whereas 90 % of counts from 2007 were greater than 10^8 CFU g^{-1} in DRBC and DG18 (Tables 2, 3 and 4). The same sampling scheme was used to collect material from the bunks in both periods. Fungal contamination was never homogeneous, existing highly contaminated hotspots surrounded by non-contaminated material. In addition, climatic and environmental variations between sampling periods may have contributed to this difference. However, a high percentage of samples in both cases (92 % of samples collected in 2006 and 93.3 % of samples collected in 2007) exceeded the limit of fungal colonies established as a hygienic quality standard – 1 x 10^4 CFU g^{-1} (GMP 2008). The results obtained in this study are comparable to other researchers' such as Abarca *et al.* (1994) - who found total counts that varied from 10^2 to 10^8 CFU g^{-1} in mixed feeds intended for cattle, swine and rabbits in Spain – and to other studies of our research group in different feedstuff samples intended for chicken, swine, cattle and horses in Argentina and Brazil, where similar counts and similar fungal species

Table 2.
Analysis of variance (ANOVA) of total fungal count means in different sampling periods and culture media

Source	GL	CM	F	P
Model	3	22.05	7.92	0.0001
Sampling year (A)	1	62.01	22.27	<0.0001
Culture media (M)	1	1.76	0.63	0.4297
A x M	1	1.36	1.36	0.4874

Table 3.
Total mixed rations' (TMR) total fungal count means and Fisher's protected LSD test.

TMR		Total fungal counts (log_{10} CFU g^{-1})	
		Mean ± SE	LSD
Year	2006	5.48 ± 0.248	*
	2007	7.29 ± 0.30	**
Media	DRBC[a]	6.54 ± 0.27	*
	DG18[b]	6.23 ± 0.27	*

Asterisks indicate statistically significant difference (*:p<0.05; **:p<0.0001).
[a]Dichloran rose bengal chloramphenicol agar.
[b]Dichloran 18 % glycerol agar.

Table 4.
Total fungal counts (CFU g^{-1}) on DRBC culture medium of cattle TMRs in different sampling periods

Sampling period	Range (CFU g^{-1})	90 Percentile[a]	Over regulation limits (%)[b]
2006	ND[c] - 1.1 x 10^8	> 2.97 x 10^7	92
2007	1.1 x 10^2 – 2.1 x 10^8	> 1.03 x 10^8	93.3

[a]90° Percentile: 90 of the samples are over this value.
[b]1 x 10^4 CFU g^{-1} as recommended by Good Manufacture Practices (GMP, 2008).
[c]ND: Not detectable (detection limit: 1 x 10^3 CFU g^{-1}).

were encountered (Dalcero *et al*., 1997; Dalcero *et al*., 1998; Magnoli *et al*., 2002; Rosa *et al*., 2006; Keller *et al*., 2007; Keller *et al*., 2008; González Pereyra *et al*., 2008a; González Pereyra *et al*., 2008b; González Pereyra *et al*., 2009).

Identification of the mycobiota revealed the presence of seven different fungal genera from feed samples in DRBC and DG18 media during the two different analyzed sampling periods. Yeasts and the order Mucorales were also isolated in both sampling periods. Yeasts prevailed at both tested sampling periods, being higher in 2007 than in 2006. Regarding to filamentous fungi, *Aspergillus* spp (60 %) was the most frequently isolated genera during 2006 followed by *Fusarium* spp (40 %), *Geotrichum* spp (40 %), *Penicillium* spp (22.5 %), Mucorales (16 %) and *Eurotium* spp (12.5 %). During 2007, *Fusarium* spp (66.7) prevailed over other genera followed by *Penicillium* spp (40 %), *Aspergillus* spp (33.3 %), *Eurotium* spp (26.7 %), *Cladosporium* spp (26.7 %), *Geotrichum* spp (6.7 %) and Mucorales (6.7 %). When considering the total of samples collected during the two years period, genera that included the main

potentially toxigenic species showed the highest isolation frequency, being *Fusarium* spp the most frequently isolated genera (50 %) followed by *Aspergillus* spp and *Penicillium* spp with very similar frequencies (Table 5).

In general, yeasts, *Aspergillus* spp*, Penicillium* spp and *Fusarium* spp prevailed in TMRs. Similar results were obtained in different studies performed on the feedstuffs mentioned above (González Pereyra *et al*., 2008a; González Pereyra *et al*., 2008b; González Pereyra *et al*., 2009). The importance of the isolation of these genera from commodities and feedstuffs relies in the fact that they include the main mycotoxigenic species.

Table 5.
Isolation frequency (%) of the different fungi isolated on DRBC and DG18 culture media from cattle TMRs

Fungi	Sampling period		Total
	2006	2007	
Aspergillus spp.	60.0	33.4	37.5
Fusarium spp.	40.0	66.7	50.0
Penicillium spp.	22.5	40.0	35.0
Eurotium spp.	15.0	26.7	25.0
Cladosporium spp.	ND	26.7	10.0
Geotrichum spp.	40.0	6.7	27.5
Alternaria spp.	12.0	ND	7.5
Mucorales	16.0	6.7	12.5
Yeasts	68.0	100.0	80.0

Aspergillus fumigatus - potential gliotoxin producer and causative of respiratory disease -was the dominant *Aspergillus* spp constituting 51.6 % of the total of isolates. Other *Aspergilli* such as *A. flavus* were also isolated (Figure 1 A). *Penicillium griseofulvum* (37.5 %) was the prevalent species from *Penicillium* genus, among other species such as *P. roqueforti, P. crustosum* and *P. brevicompactum* (Figure 1 B). The prevalent *Fusarium* spp, was *F. verticillioides* (60 %) followed by *F. proliferatum* (20 %) and *F. subglutinan*s (20 %) (Figure 1 C).

The presence of the major toxigenic species *A. flavus* is another matter of concern. Moreover, half of the isolates were able to produce AFB_1. The presence of aflatoxigenic strains in feedstuffs constitutes a potential risk for animal health and productivity since the toxin can be produced in the substrate if exposed to inadequate storing conditions. This was confirmed by the presence of AFB_1 contamination in some of the samples collected during 2007. *Penicillium griseofulvum* (potential patulin and roquefortine C producer), *P. roqueforti* and *P. crustosum* (potential roquefortine C producers) were also isolated with high frequency. These species were also found in the corn silage used as an ingredient of the TMR of one of the farms (González Pereyra *et al*., 2011) and by other authors in the same kind of commodity (O'Brien *et al*., 2005; Garon *et al*., 2006; Richard *et al*, 2007). Three *Fusarium* spp were isolated from cattle feed samples, being *F. verticillioides* the dominant species like has been reported by other authors in several feedstuffs (Garon *et al*., 2006; Richard *et al*., 2007).

Mycotoxigenic capacity of strains

When evaluating ability of *A. flavus* strains to produce AFs, 50 % of the isolates produced AFB_1 levels ranging from 75 to 112.5 μg g^{-1}

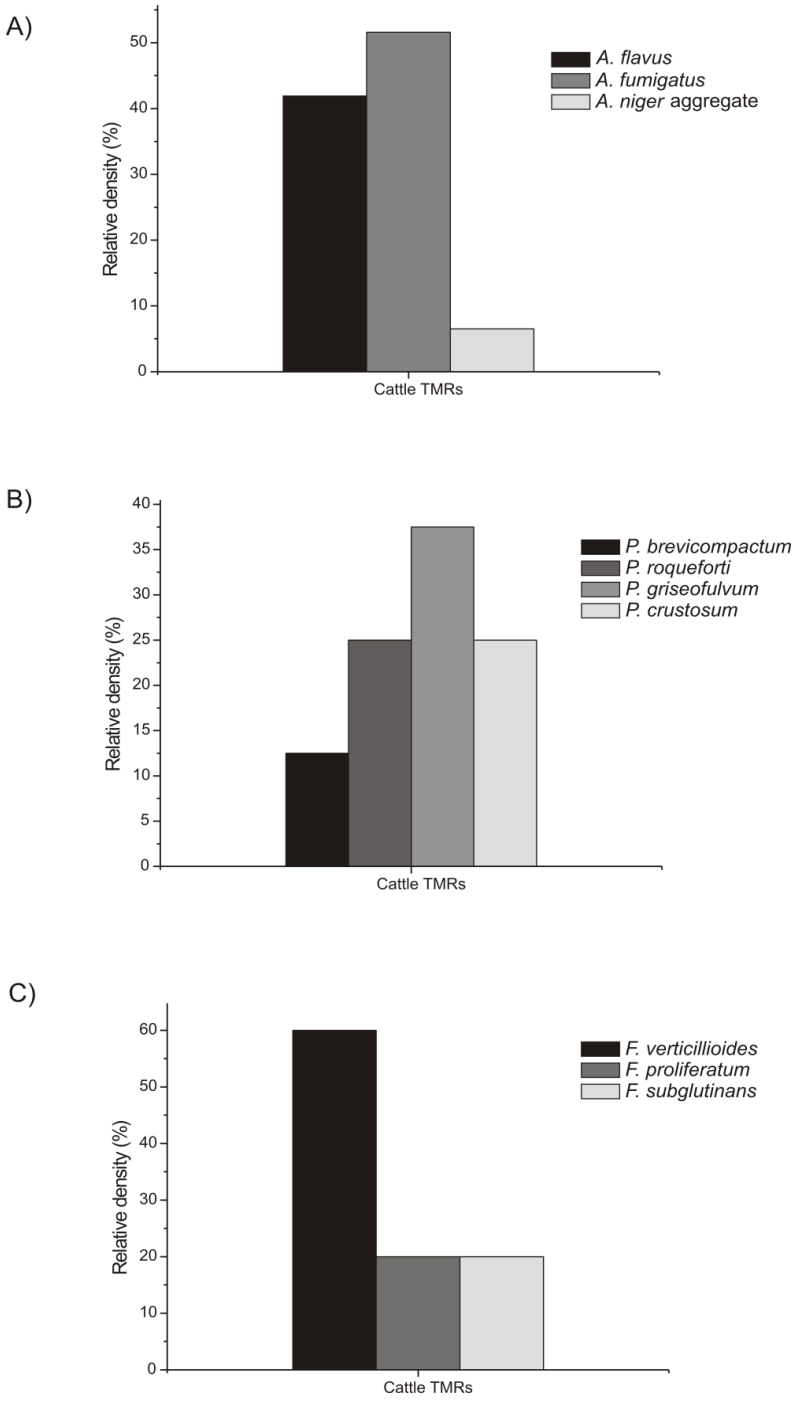

Figure 1. Relative density (%) of a) *Aspergillus* and b) *Penicillium* species isolated on malt extract agar (MEA) and c) *Fusarium* species isolated on carnation leaf agar (CLA) from cattle total mixed rations (TMRs).

(ppm). The remaining 50 % of the strains did not produce AFs *in vitro*. Aflatoxin B_2, AFG_1 and AFG_2 were not produced by any of the tested strains.

Mycotoxin Analyses

Samples collected in 2006, did not show detectable levels of mycotoxins. Aflatoxin B_1 was detected in 46.7 % of cattle feed samples collected during 2007 with levels ranging from 4 to 10 μg kg^{-1} . Deoxynivalenol was detected in 33.3 % of these samples and levels were ≥1.25 μg g^{-1} (detection limit 0.5 μg g^{-1}). Fumonisin B_1 and ZEA levels were under the detection limits of the used methodologies in all analyzed samples (Table 6).

The presence of the major toxigenic species - *A. flavus* - is a matter of concern. Moreover, half of the isolates were able to produce AFB_1. The presence of aflatoxigenic strains in feedstuffs constitutes a potential risk for animal health and productivity since the toxin can be produced in the substrate if exposed to inadequate storing conditions. This was confirmed by the presence of AFB_1 contamination in some of the samples collected during 2007. *Penicillium griseofulvum* (potential patulin and roquefortine C producer), *P. roqueforti* and *P. crustosum* (potential roquefortine C producers) were also isolated with high frequency. These species were also found in the corn silage used as an ingredient of the TMR of one of the farms (González Pereyra *et al*., 2011) and by other authors in the same kind of commodity (O'Brien *et al.*, 2005; Garon *et al*., 2006; Richard *et al.*, 2007). Three *Fusarium* spp were isolated from cattle feed samples, being *F. verticillioides* the dominant species like has been reported by other authors in several feedstuffs (Garon *et al*., 2006; Richard *et al*., 2007).

Studying the pre-existing mycobiota in a given commodity can sometimes be used as a guideline to estimate the mycotoxins that could potentially be contaminating the substrate (González Pereyra *et al*., 2008a). However, mycotoxins are more resistant than mycelia to the feedstuffs processing and storing conditions for they can be found in samples where the mould can no longer be isolated. In the present study, mycotoxins were detected in low frequency and only in samples collected during 2007. In the case of AFB_1, levels were below the recommended limit for animal feeds feed ingredients intended for beef cattle (20 μg kg^{-1}) (GMP 2008). Deoxynivalenol content was determined by an immunochromatographic technique due to the lack of an appropriate HPLC method for

Table 6.
Mycotoxins levels detected in cattle TMRs at different sampling periods

Mycotoxin	Sampling period	Contaminated samples		Levels (μg g^{-1})
		N°	%	
AFB_1	2006	0	0	ND[a]
	2007	8	53.30	4
		2	13.33	10
DON	2006	0	0	ND
	2007	5	33.33	≥ 1250[b]
FB_1	2006	0	0	ND
	2007	0	0	ND
ZEA	2006	0	0	ND
	2007	0	0	ND

[a]ND: not detectable.
[b]Semi-quantitative result according to RIDA®QUICK DON Immunochromatographic Test kit.

this kind of substrate. The method we use for wheat (Cooney *et al*., 2001), corn and other grains was not suitable for TMRs since much interference was seen in the chromatograms. Total mixed rations are complex matrixes and obtaining clean extracts for HPLC mycotoxin detection is often difficult. Fumonisin B_1 and zearalenone were not detected in any of the analyzed samples even though fusarium potentially producer species were present.

Results reported in the present study contribute to the learning of mycobiota and the natural mycotoxin contamination present in TMRs intended for feedlot cattle used in one of the main beef-producing regions of Argentina. The information collected here can be useful to estimate the mycotoxicological risk in TMRs, since most studies report information on corn, silage or other feedstuffs or ingredients but not on the finished product as it is consumed by the animals, collected directly from the bunks.

We conclude that toxigenic and potentially toxigenic species are found regularly in cattle TMRs. If storage conditions of the different feedstuffs used for TMRs formulation are adequate for mycotoxin production, the contamination of the final product will be imminent. The levels of toxin can be variable and, in consecuence, so will be the concentration of mycotoxins in the final product. Monitoring this substrate during two consecutive years led us to the conclusion that periodical analyses are needed for fungal and consequently mycotoxin contamination are highly heterogeneous in their distribution and levels may change from year to year.

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