

# FUNGAL AND MYCOTOXIN CONTAMINATION IN MIXED FEEDS: EVALUATING RISK IN CATTLE INTENSIVE REARING OPERATIONS (FEEDLOTS)\*

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**ABSTRACT.** Gonzales Pereyra M.L., Chiacchiera S.M., Rosa C.A. da R., Sager R., Dalcero A.M. & Cavaglieri L.R. **Fungal and mycotoxin contamination in mixed feeds: Evaluating risk in cattle intensive rearing operations (Feedlots).** [Contaminação por fungos e micotoxinas em rações para bovinos: Avaliação de risco em operações de criação intensiva de gado (confinamento)]. *Revista Brasileira de Medicina Veterinária*, 34(4):311-318, 2012. Departamento de Microbiología e Inmunología, Universidad Nacional de Río Cuarto, Ruta 36 Km, 601, (5800) Río Cuarto, Córdoba, Argentina. E-mail: lcavaglieri@exa.unrc.edu.ar

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<sub>1</sub> (AFB<sub>1</sub>), fumonisin B<sub>1</sub> (FB<sub>1</sub>), 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<sub>1</sub> *in vitro* was tested. Natural contamination with AFB<sub>1</sub> and FB<sub>1</sub> was quantified by HPLC. Deoxynivalenol and zearalenone were analysed by immunochromatography and TLC, respectively. Fungal counts varied from ND to 2.10 x 10<sup>8</sup> CFU/g. The prevalent genera were *Aspergillus* spp. (60%) and *Fusarium* spp. (66.7%), respectively. The prevalent species was *Aspergillus fumigatus*. Fifty percent of *A. flavus* strains produced 75 to 112.5 µg/g AFB<sub>1</sub>. Forty-six percent of 2007 samples were contaminated with 4 to 10 µg/kg AFB<sub>1</sub>. Deoxynivalenol was detected in 33.3 % of the samples (≥ 1.25 µg/g). Fumonisin B<sub>1</sub> 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.

**RESUMO.** A Argentina é a quarta maior produtora mundial de carne. Com isso, a exposição às micotoxinas através de alimentos contaminados é um grande risco para o gado. No presente estudo a micobiota e a contaminação natural com aflatoxi-

na B<sub>1</sub> (AFB<sub>1</sub>), fumonisina B<sub>1</sub> (FB<sub>1</sub>), deoxinivalenol (DON) e zearalenona (ZEA) em rações completas mistas (TMRs) para bovinos foram avaliadas durante dois anos consecutivos. As contagens totais de fungos (UFC/g) foram avaliadas e as espécies

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fúngicas foram identificadas. A capacidade dos *A. flavus* isolados para produzir AFB<sub>1</sub> *in vitro* e a contaminação das rações com AFB<sub>1</sub>, FB<sub>1</sub>, DON e ZEA foram testadas. As contagens totais de fungos variaram entre não detectado (ND) e 2,10x10<sup>8</sup> UFC/g. *Aspergillus* spp. (60%) e *Fusarium* spp. (66,7%) foram os gêneros prevalentes, enquanto *A. fumigatus* foi a espécie isolada com maior frequência. Cinquenta por cento dos *A. flavus* isolados foram produtores de 75 a 112,5 µg/g de AFB<sub>1</sub>. Quarenta e seis por cento das amostras coletadas em 2007 estavam contaminadas com 4 a 10 µg/kg AFB<sub>1</sub>. Deoxinivalenol foi detectado em 33,3% das amostras (≥ 1,25 µg/g), enquanto FB<sub>1</sub> e ZEA não foram detectadas. Este estudo é útil para estimar o risco de micotoxicológico das TMRs usadas na produção intensiva de gado numa das regiões produtoras de carne bovina mais importante e comparar os resultados com estudos de outros países.

**PALAVRAS-CHAVE.** Aflatoxinas, deoxinivalenol, fumonisi-na, gado, rações.

## 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 *Aspergillus*, *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<sub>1</sub> can induce the presence of aflatoxin M<sub>1</sub> 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). Fumonisin (FBs) have been associated to the occurrence of pulmonary edema in pigs, leuko-

encephalomalacia 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<sub>2</sub> 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, 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 very 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<sub>1</sub>, FB<sub>1</sub>, 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 lo-

cated 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  $39^{\circ}\text{C}$  with an annual average of  $17^{\circ}\text{C}$ . 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 kg 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  $65^{\circ}\text{C}$ , milled and stored at  $4^{\circ}\text{C}$  until mycotoxin analyses.

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 <sup>a</sup>
Concentrate (monensine, urea and minerals)	1.5	Concentrate (monensine, urea and minerals)	5
Soybean or wheat pellet	3.5		

<sup>a</sup>Adequate amounts were added to the diet in order to reach 10% acid detergent fiber (ADF) content.

### 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 ISO 21527-2. Ten grams 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  $25^{\circ}\text{C}$  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). 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 & 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<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub> in MEA medium *in vitro* was tested according to methodology described by Geisen et al. (1996). Five day cultures growing in MEA at  $30^{\circ}\text{C}$  were extracted with 500  $\mu\text{l}$  chloroform and centrifuged 20 min at 3000 rpm. The mycelia was removed, the extracts were evaporated under N<sub>2</sub> flow and re-dissolved in 200  $\mu\text{l}$  chloroform. Two, 5 and 10  $\mu\text{l}$  drops were spotted on silica gel 60 TLC aluminum sheets (20 x 20 cm, Merck™, Germany) along with 2, 5 and 10  $\mu\text{l}$  spots of a quantified aflatoxin standard solution (containing AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>). 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<sub>1</sub> 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<sub>2</sub> flow and redissolved in 400  $\mu\text{l}$  mobile phase acetonitrile:methanol:water (17:17:66, v/v). An aliquot (200  $\mu\text{l}$ ) was derivatized with 700  $\mu\text{l}$  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.

Fumonisin B<sub>1</sub> content of TMRs was determined as follows: 25 g of feed were ground with a grinder (20 mesh; 840  $\mu\text{m}$  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 N° 4 paper 4 (Whatman, Inc., Clifton, New Jersey, USA). The extracts were analysed for FB<sub>1</sub> by HPLC using the method proposed by Shephard et al. (1990) and modified by Doko et al. (1995). For HPLC analysis of FB<sub>1</sub> 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. Twenty 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 dihydrogenated sodium phosphate (75: 25) solution, pH 3. 35. Flow was fixed to 1.5 ml/min. Fumonisin B<sub>1</sub> was quantified by comparing peak height measurements with a reference standard solution. The standard solution was obtained by dissolving crystalline FB<sub>1</sub> (Division of Food Science and Technology, Pretoria, South Africa) in acetonitrile:water (1:1). A five point standard curve was developed to quantify FB<sub>1</sub>. Quantification limit of the method was 20 ng/g.

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.

Zearalenone analysis was performed by thin layer chromatography (TLC) as it is described in the Official Methods of Analysis (AOAC, 1995). The extracts were screened for ZEA contamination by spotting 2 µl, 5 µl, 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.

### Statistical analyses

Data analyses were performed by analysis of variance (ANOVA). Fungal counts data were transformed using the logarithmical function  $\log_{10}(x + 1)$  before applying ANOVA. Duncan's test was

used for comparing CFU/g 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

### Analysis of the mycobiota

Total fungal counts present in cattle TMRs varied from less than  $1 \times 10^3$  CFU/g (detection limit of the method) to  $2.10 \times 10^8$  CFU/g in DRBC and from ND to  $1.8 \times 10^8$  CFU/g in DG18. Ninety-two percent of samples collected during 2006 and 93.3 % of samples collected in 2007 exceeded the limit of fungal colonies established as a hygienic quality standard ( $1 \times 10^4$  CFU/g) according to Good Manufacture Practices guidance (GMP 2008). When comparing both DRBC and DG18 counts of the different sampling periods, higher counts could be observed during 2007. Ninety percent of counts from 2006 were greater than  $10^7$  CFU/g in DRBC and  $10^6$  CFU/g in DG18 whereas 90% of counts from 2007 were greater than  $10^8$  CFU/g in DRBC and DG18 (Tables 2, 3 and 4).

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		22,27	<0,0001
Culture media (M)	1		0,63	0,4297
A x M	1		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)		
	Mean ± SE	LSD (p < 0,05)	
Year	2006	5,48 ± 0,248	*
	2007	7,29 ± 0,30	**
Media	DRBC <sup>a</sup>	6,54 ± 0,27	*
	DG18 <sup>b</sup>	6,23 ± 0,27	*

Asterisks indicate statistically significant difference (p<0,05).

<sup>a</sup>Dichloran rose bengal chloramphenicol agar.

<sup>b</sup>Dichloran 18% glycerol agar.

Table 4. Total fungal counts (CFU/g) on DRBC culture medium of cattle TMRs in different sampling periods

Sampling period	Range (CFU/g)	90 Percentile <sup>a</sup>	Over regulation limits (%) <sup>b</sup>
2006	ND <sup>c</sup> - $1.1 \times 10^8$	$> 2.97 \times 10^7$	92
2007	$1.1 \times 10^2$ - $2.1 \times 10^8$	$> 1.03 \times 10^8$	93.3

<sup>a</sup>90° Percentile: 90 % of the samples are over this value.

<sup>b</sup> $1 \times 10^4$  CFU/g as recommended by Good Manufacture Practices (GMP, 2008).

<sup>c</sup>ND: Not detectable (detection limit:  $1 \times 10^3$  CFU/g).

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).

*Aspergillus fumigatus* was the dominant *Aspergillus* species, constituting 51.6% of the total of isolates. Other aspergilli such as *A. flavus* and *A. fumigatus* were also isolated (Figure 1a). *Penicillium griseofulvum* (37.5%) was the prevalent species from *Penicillium* genus, among other species such as *P. roqueforti*, *P. crustosum* and *P. brevicompactum* (Figure 1b). The prevalent *Fusarium* species was *F. verticillioides* (60%) followed by *F. proliferatum* (20%) and *F. subglutinans* (20%) (Figure 1c).

### Mycotoxigenic capacity of strains

When evaluating ability of *A. flavus* strains to produce AFs, 50 % of the isolates produced AFB<sub>1</sub> levels ranging from 75 to 112.5 µg/g (ppm). The remaining 50 % of the strains did not produce AFs

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

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

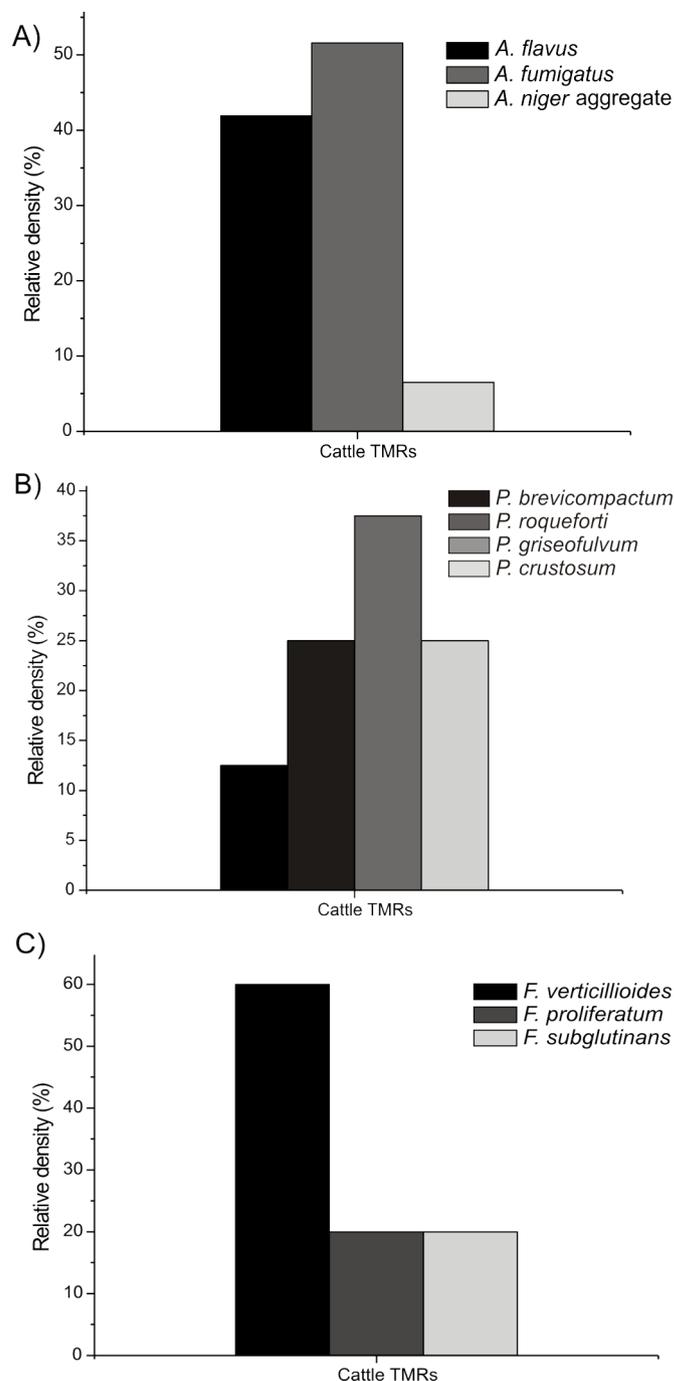


Figure 1. Relative density (%) of a) *Aspergillus* and b) *Penicillium* species isolated on malt extract agar (MEA) and c) *Fusarium* species isolated on carnation leaf agar (CLA) from cattle total mixed rations (TMRs).

*in vitro*. Aflatoxin B<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub> were not produced by any of the tested strains.

### Mycotoxin Analyses

Samples collected in 2006, did not show detectable levels of mycotoxins. Aflatoxin B<sub>1</sub> was detected in 46.7 % of cattle feed samples collected during 2007 with levels ranging from 4 to 10 µg/kg. Deoxynivalenol was detected in 33.3 % of these sam-

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

Mycotoxin	Sampling period	Contaminated samples		Levels ( $\mu\text{g/g}$ )
		N <sup>o</sup>	%	
AFB <sub>1</sub>	2006	0	0	ND <sup>a</sup>
	2007	8	53.30	4
		2	13.33	10
DON	2006	0	0	ND
	2007	5	33.33	$\geq 1250^b$
FB <sub>1</sub>	2006	0	0	ND
	2007	0	0	ND
ZEA	2006	0	0	ND
	2007	0	0	ND

<sup>a</sup>ND: not detectable.

<sup>b</sup>Semi-quantitative result according to RIDA@QUICK DON Immunochromatographic Test kit.

ples and levels were  $\geq 1.25 \mu\text{g/g}$  (detection limit  $0.5 \mu\text{g/g}$ ). Fumonisin B<sub>1</sub> and ZEA levels were under the detection limits of the used methodologies in all analyzed samples (Table 6).

## DISCUSSION

The samples for this study were collected from different feed-lots located in one of the most important regions of Argentina where an intensive cattle rearing is developed. Total fungal counts of samples collected in 2007 were higher than those of samples collected in 2006. 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 exceeded the limit of fungal colonies established as a hygienic quality standard –  $1 \times 10^4$  CFU/g (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 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 were encountered (Dalcero et al. 1997, 1998, Magnoli et al. 2002, Rosa et al. 2006, Keller et al. 2007, 2008, González Pereyra et al. 2008a,b, 2009).

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,b, 2009). The importance of the

isolation of these genera from commodities and feedstuffs relies in the fact that they include the main mycotoxigenic species. *Aspergillus fumigatus*, potential gliotoxin producer and causative of respiratory disease, was the dominant species present in these samples. The presence of the major toxigenic species *A. flavus* is another matter of concern. Moreover, half of the isolates were able to produce AFB<sub>1</sub>. 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<sub>1</sub> 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* species 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<sub>1</sub>, levels were below the recommended limit for animal feeds feed ingredients intended for beef cattle ( $20 \mu\text{g/kg}$ ) (GMP 2008). Deoxynivalenol content was determined by an immunochromatographic technique due to the lack of an appropriate HPLC method for 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<sub>1</sub> 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.

## CONCLUSION

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 consequence, 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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