

CHRONIC EXPOSURE TO AFLATOXIGENIC FUNGI RELATED TO LIVER DAMAGE IN PELT CHINCHILLAS (*Chinchilla lanigera*)*

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ABSTRACT. Landa M.F., González Pereyra M.L., Pena G., Bagnis G., Cavaglieri L.R., Rosa C.A.R. & Dalcerro A.M. **Chronic exposure to aflatoxigenic fungi related to liver damage in pelt chinchillas (*Chinchilla lanigera*).** [Exposição crônica a fungos produtores de aflatoxinas relacionada a danos hepáticos em chinchilas (*Chinchilla lanigera*) de pele]. *Revista Brasileira de Medicina Veterinária*, 34(4):303-310, 2012. Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta N 36 Km 601 (5800), Río Cuarto, Córdoba, Argentina. E.mail: adalcerro@exa.unrc.edu.ar

Chinchilla pelt is a rare and expensive fur. Therefore, breeding these animals is a profitable activity. Confirmed acute cases of aflatoxin intoxication have been reported in Argentinean farms. The aims of this study were i) to evaluate mycobiota and AFB₁-producing species in chinchilla feeds ii) to investigate their natural AFB₁ contamination and iii) to analyze histopathological lesions in chinchilla livers. Feed samples (A: fur, B: mother, C: lucerne cubes) were collected from a factory and a farm. Livers of sacrificed chinchilla from the farm were macroscopically and microscopically examined. Total fungal counts of feed C exceeded 1x10⁴ CFU/g. *Aspergillus*, *Fusarium* and *Penicillium* were the prevalent genera, while *A. flavus*, *A. fumigatus*, *F. verticillioides* and *F. proliferatum* were the prevalent species. Fifty % of *A. flavus* strains from factory samples and 69.7% from farm samples were able to produce 2.78 to 8.64µg/g and 0.66 to 58.8µg/g AFB₁ respectively. Aflatoxin B₁ was detected only in feeds from the farm, finding the highest incidence in feed C. Toxin levels varied between 1.90 and 97.34 µg/kg AFB₁. Mean levels in feed A and C exceeded 20 µg/kg. Macroscopic examination of livers revealed normal appearance, size and color. However, histopathological examination indicated 63.3% showed slight to moderate lipid degeneration with diffuse cytoplasm vacuolation, 9% intense lipid cytoplasm vacuolation and 27.3% hydropic degeneration and nuclear vacuolation in hepatocytes. A periodic monitoring of aflatoxins in feeds and their ingredients can prevent acute outbreaks and economic losses caused by chronic exposure.

KEY WORD. Aflatoxins, *Aspergillus* spp., *Chinchilla lanigera*, feed, histopathology, toxicology.

RESUMO. A pele de chinchila é uma das mais exóticas e caras. Portanto, a criação destes animais é uma atividade rentável. Casos agudos de intoxi-

cação por aflatoxinas têm sido informados em fazendas Argentinas. Os objetivos deste estudo foram: i) Avaliar a micobiota e os fungos produtores

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de Aflatoxina B₁ (AFB₁) presente em rações para chinchilas ii) analisar a contaminação natural com AFB₁ destas rações e iii) pesquisar lesões histopatológicas nos fígados das chinchilas. As amostras de diferentes rações (A: pele, B: mãe, C: cubos de alfafa) foram coletadas numa fábrica e uma fazenda localizadas na cidade de Rio Cuarto, na região central da Argentina. Os fígados das chinchilas eutanasiadas, na fazenda, foram analisados macroscópica e microscopicamente. As contagens totais de fungos das rações foram superiores a 1x10⁴ UFC/g. *Aspergillus*, *Fusarium* e *Penicillium* foram os gêneros prevalentes, enquanto *A. flavus*, *A. fumigatus*, *F. verticillioides* e *F. proliferatum* foram às espécies isoladas com maior frequência. Cinquenta por cento dos *A. flavus* isolados da fábrica e 69,7% dos isolados da fazenda foram produtores de 2,78 a 8,64 µg/g e 0,66 a 58,8 µg/g de AFB₁, respectivamente. A AFB₁ foi detectada só nas amostras coletadas na fazenda, com maior incidência na ração C. Os níveis de toxina variaram entre 1,90 e 97,34 µg/kg. Os níveis médios na ração A e C foram superiores a 20 µg/kg. Análise macroscópica dos fígados revelou aparência, tamanho e cor normais. No entanto, o exame histopatológico indicou que 63,3% dos fígados apresentaram leve a moderada degeneração lipídica com vacuolização difusa do citoplasma, 9% apresentaram vacuolização lipídica intensa do citoplasma e 27,3% apresentaram degeneração hidrópica e vacuolização nuclear nos hepatócitos. O monitoramento periódico da qualidade das rações e ingredientes usados na alimentação de chinchilas pode evitar intoxicações agudas e perdas econômicas causadas pela exposição crônica as aflatoxinas.

PALAVRAS-CHAVE. Aflatoxinas, *Aspergillus* spp., *Chinchilla lanigera*, histopatologia, toxicologia.

INTRODUCTION

Fungal contamination of harvest products and animal feeds with fungi and mycotoxins is a worldwide concern. Mycotoxins have been detected in several commodities, ingredients and final products destined to animal feeding throughout the world (Whitlow & Hagler 2002, Monbaliu et al. 2010).

Aflatoxins (AFs) are secondary metabolites produced by toxigenic strains of *Aspergillus flavus* and *A. parasiticus*. Chemically, AFs belong to the group of bifurano coumarins, being aflatoxins B₁ (AFB₁), the most toxic and hazardous one. Aflatoxin B₁ is hepatotoxic, highly mutagenic, carcinogenic and probably teratogenic to animals (Smith & Moss

1985). Aflatoxin B₁ has been classified as a class 1 human carcinogen by the International Agency for Research on Cancer (IARC 2002).

In all species, the liver is the primary target organ of acute injury caused by aflatoxins (AFs). The first step in the biotransformation of AFB₁ takes place in the hepatocyte, with nonreversible detoxification via the formation of hydroxylated metabolites (AFM₁, AFQ₁, AFP₁, AFB₂), followed either by reversible detoxification through aflatoxicol formation, or by activation through the generation of AFB₁-8,9-epoxide (Neal 1998). Acute aflatoxicosis is frequently associated with the ingestion of large doses of AFs, which cause typical hepatic changes, such as liver enlargement, color change, fat accumulation, and lipid vacuolation, which are confirmed by necropsy and histopathology (CAST 2003, Newman et al. 2007).

Chinchillas (*Chinchilla lanigera*) are rabbit-sized crepuscular rodents native to the Andes Mountains in South America. At the present time, this species practically no longer exists in its natural habitat. Chinchillas are raised in farm for fur and pets. Currently, chinchilla pelt along with Russian sable, are the most prestigious, rare and expensive furs. The growing international demand for chinchilla fur makes the breeding of these animals a highly profitable activity in Argentina (González Pereyra et al. 2008a).

Chinchillas are known to be very sensitive to mycotoxins, and a large number of animals often die when acute aflatoxicosis occurs. Clinical signs that may indicate mycotoxicosis include low feed intake, diarrhea, weight loss, poor condition of the skin, fur discoloration, sudden death, and a predisposition to secondary infections (Labala 2008). Confirmed cases of acute aflatoxin intoxication have been reported in Argentina (González Pereyra et al. 2008a). However, the mycobiota of chinchilla feeds and the identification of AFs producer species have not been reported yet. Since chinchillas are rare, expensive and very delicate animals, the mycotoxin content of their feed should be reduced to the minimum to avoid death, immunosuppression and fur loss. The use of quality feeds and feed ingredients is a key to minimize economic losses.

The aims of the present study were i) to evaluate the mycobiota and the presence of AFB₁ producer species in chinchilla feeds ii) to investigate the incidence of natural AFB₁ contamination in these feeds and iii) to analyze histopathological lesions in chin-

chilla livers in search for typical changes associated to aflatoxin exposure.

MATERIALS AND METHODS

Source of samples

A total of 77 chinchilla feed samples (5 kg each) were collected during twelve months (June 2009 to June 2010) from two different sources: a chinchilla farm and a feed factory both located in Rio Cuarto city, Córdoba Province, Argentina. Three samples of different chinchilla feeds were collected monthly: A) fur chinchillas, B) mother chinchillas and C) lucerne cubes. The latter was sampled only in the chinchilla farm, since it was not produced in the factory. The samples were homogenized and quartered to obtain a 1 kg laboratory sample. Water activity (a_w) of the samples was measured using an AQUALAB CX2 (Decagon, Devices, Inc. USA) appliance. A 20 g aliquot from each was randomly selected for the analysis of the mycobiota and the rest was stored at 4°C until mycotoxin analysis.

Mycological survey

Total fungal counts were performed on two different culture media: dichloran rose bengal chloranphenicol agar (DRBC) for estimating total mycobiota (Abarca et al. 1997 - ISO 21527-1) and dichloran 18% glycerol agar (Pitt & Hocking 1997 - DG18) to favor xerophilic fungi development (ISO 21527-2). Quantitative enumeration was done using the plate count method. Twenty grams of each sample were homogenized in 180 ml 0.1% peptone water solution for 30 min. Serial dilutions (10^{-2} to 10^{-4}) were made and 0.1 ml aliquots were inoculated in triplicates on the solid media. After 7 days of incubation at 25°C, plates containing 10–100 CFU were used for counting and the results were expressed as CFU per gram of sample (CFU/g). On the last day of incubation, individual CFU/g counts for each colony type considered to be different were recorded. Colonies representative of each type were transferred to plates with malt extract agar (MEA). Fungal colonies were selected for identification, according to Pitt & Hocking (1997), Klich (2002) and Nelson et al. (1983), depending on the genus. The results were expressed as isolation frequency (percentage of samples in which each genus/species was present) and relative density (percentage of isolates of the same species among the total number of isolates of a certain genus).

Ability of *Aspergillus section flavi* to produce AFB₁ in vitro

Ability of 20 *Aspergillus section flavi* strains to produce AFB₁ *in vitro* was tested by thin layer chromatography (TLC) (Geisen, 1996). Strains were inoculated on MEA and incubated for 7 days at 25 °C. Mycelium was transferred to previously weighted Eppendorff tubes. Mycelium weight was calculated by weight difference. Five hundred µl of chloroform were added and the mixture was shaken for 20 min at 400 rpm. The mycelial mass was removed and the chloroform extract was evaporated under N₂ flow. Five µl of each sample extract were spotted on silica gel TLC plates (Merk, Germany), 2 cm from the bottom edge. Different volumes of AFB₁ standard solution were spotted in each plate along with the extracts. Plates were developed in chloroform:acetone (9:1 v/v) at room temperature. When the solvent front was 15 cm from the spot line, the plates were removed and dried to room temperature. Plates were examined under 365 nm UV light for the quantification of AFB₁ through visual comparison with the standard solution of known concentration. Limit of detection (LOD) of this method was 5 µg/kg.

Aflatoxin analysis of feed samples

AFB₁ analysis was performed by HPLC according to Trucksess et al. (1994). For each sample, 25 g of chinchilla feed were extracted with 100 ml acetonitrile: water (84:16, v/v). The mixture was shaken for 30 min in an orbital shaker and filtered through Whatman N°4 filter paper (Whatman, Inc., Clifton, New Jersey, USA). An 8 ml aliquot was taken and placed into a 10 ml culture tube. An Afla-Pat Mycosep®228 clean-up column (Romer Labs Inc., Union, MO, USA) multifunctional column was used to obtain a purified extract (4 ml) that was collected and evaporated under N₂ flow. Extracts were resuspended in 400 µl Water:methanol:acetonitrile (4:1:1, v/v). Aliquots of 200 µl were derivatized with 700 µl trifluoroacetic acid:acetic acid:water (20:10:70, v/v). The derivatized extracts were analyzed by using an HPLC system. Chromatographic separations were performed on a C18 RP Phenomenex Luna (150 x 4.60 mm, 5 µ) column (Phenomenex, USA). A Water: methanol: acetonitrile (4:1:1, v/v) solution was used as mobile phase at 1 ml/min flow rate. Fluorescence of aflatoxin derivatives was recorded at 360 nm excitation 460 nm emission wavelengths. Standard curves were cons-

tructed using AFB₁ standard solutions of different concentration. The toxin was quantified by correlating peak heights of sample extracts with those of standard solutions in a calibration curve. The LOD of this method was 1 ng/g.

Histopathological examination

Fifteen chinchilla livers were obtained from the farm. All animals had been sacrificed for fur. Macroscopic characteristics of the organs were evaluated. These included general size (lateral width), weight, and color. Liver tissue for histological analysis was fixed in 10% neutral buffered formalin and trimmed. They were processed routinely, embedded in paraffin, sectioned at 5-mm thickness, and stained with hematoxylin and eosin (H/E). An histopathological analysis was performed to evaluate hepatocellular characteristics and lesions such as cytoplasmic vacuolation, nodular hyperplasia, and bile-duct proliferation (CAST 2003, Allameh et al. 2005, Miazzo et al. 2005).

Statistical analysis

Analysis of CFU/g was performed by Mixed and General Lineal Model. Fisher's LSD test was done to compare means of treatments (Quinn & Keough 2002). The analysis was conducted using PROC GLM in SAS (SAS Institute, Cary, NC, USA).

RESULTS

Water activity values of feeds samples ranged from 0.402 to 0.613. Total fungal counts (CFU/g) of chinchilla feeds varied from 1x10² to 1.4x10⁶ (Table 1). Total fungal counts of lucerne cubes exceeded the limit established for good quality feeds and feed ingredients by the Good Manufacturing Practices (1x10⁴ CFU/g) (GMP, 2008). There was

Table 1. Fungal counts in chinchilla feeds. Total fungal counts (UFC g⁻¹) obtained from three different kinds of chinchilla feeds (A: fur chinchillas, B: mother chinchillas and C: lucerne cubes) on DRBC and DG18 media.

Source of Samples	Kind of sample	Total fungal counts (UFC g ⁻¹)		DG18
		DRBC	Contaminated samples exceeding recommended limits (%)*	
Farm	feed A	1.0 x10 ² - 1.7 x10 ⁴	0	2.0 x10 ² - 1.0 x10 ⁴
	feed B	1.0 x10 ² - 3.6 x10 ³	0	1.0 x10 ² -7.0 x10 ³
	feed C	1.0 x10 ² - 1.4 x10 ⁶	77,7	1.0 x10 ² - 6.0 x10 ⁵
Factory	feed A	2.0 x10 ² - 6.0 x10 ²	0	7.0 x10 ² - 7.0 x10 ³
	feed B	2.0 x10 ² - 3.0 x10 ³	0	2.0 x10 ² - 3.0 x10 ³

* Recommended limits established by Good Manufacturing Practices, GMP (2008).

no significant difference between counts on DRBC and DG18 media, or between the different sources of samples (farm and factory). However, there was significant difference between the different kinds of feed (A, B and C) (Tables 2 and 3).

The occurrence of *Aspergillus*, *Fusarium* and *Penicillium* genera was evaluated calculating isolation frequency. *Aspergillus* was the prevalent genera in feed A and feed B from the farm, while *Fusarium* was predominant in feed C. In feeds from the factory, the most frequent genera found was *Fusarium* followed by *Penicillium* and *Aspergillus* (Figure 1).

Aspergillus and *Fusarium* species were identified. *Aspergillus flavus*, *A. fumigatus*, *F. verticillio*

Table 2. Statistical analysis of fungal counts in chinchilla feeds. Analysis of CFU g⁻¹ by Mixed and General Lineal Model. Software: PROC GLM in SAS (SAS Institute, Cary, NC, USA).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Source of sample	1	2,45	2,45	1,30	0,2540
Kind of feed	2	297,77	148,89	79,31	<0,0001
Media	1	0,35	0,35	0,19	0,6646
Source: Kind of feed	1	4,74	4,74	2,53	0,1128
Kind of feed: Media	2	8,10	4,05	2,16	0,1168
Source: Kind of feed:Media	1	0,11	0,11	0,06	0,8114
Residuals	416	780,89	1,88		

Table 3. Statistical analysis of fungal counts in chinchilla feeds.

Kind of feed	Mean ^a	SE ^b
A	1,67 ^k	0,13
B	2,01 ^k	0,16
C	4,82 ^y	0,29

^a Fisher's LSD test to compare mean values. Different letters indicate statistically significant difference.

^b Standard error

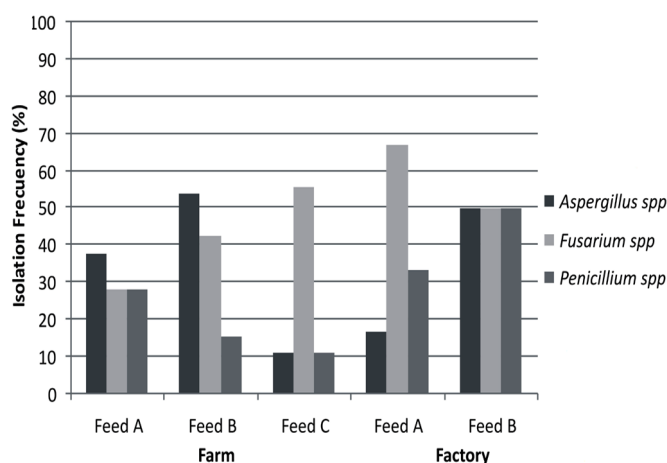


Figure 1. Isolation frequency of fungal genera identified in different kinds of chinchilla feeds (A: fur chinchillas, B: mother chinchillas and C: Lucerne cubes) from different sources (farm and factory).

Table 4. *Aspergillus* and *Fusarium* species in chinchilla feeds.

Fungal species relative density (%) ^a									
Farm					Factory				
feed A		feed B		feed C	feed A		feed B		
<i>A. flavus</i>	83,3	<i>A. flavus</i>	55,5	<i>A. flavus</i>	100	<i>A. flavus</i>	100	<i>A. flavus</i>	100
<i>A. fumigatus</i>	16,6	<i>A. fumigatus</i>	16,6	<i>F. verticillioides</i>	66,7	<i>F. verticillioides</i>	71,4	<i>F. verticillioides</i>	75
<i>F. verticillioides</i>	78,9	<i>A. versicolor</i>	22,2	<i>F. oxysporum</i>	33,4	<i>F. proliferatum</i>	28,6	<i>F. proliferatum</i>	25
<i>F. proliferatum</i>	15,8	<i>A. alliaceus</i>	15,8						
<i>F. oxysporum</i>	5,3	<i>F. verticillioides</i>	50						
		<i>F. proliferatum</i>	37,5						
		<i>F. oxysporum</i>	12,5						

^aDistribution of *Aspergillus* and *Fusarium* species in different kinds of chinchilla feeds (A: fur chinchillas, B: mother chinchillas and C: Lucerne cubes) from different sources (farm and factory).

ides and *F. proliferatum* were the most frequently isolated species in all different chinchilla feeds (Table 4).

All *Aspergillus spp.* strains isolated from the factory samples were identified as *A. flavus* and 50% of them were able to produce AFB₁ in values ranging from 2.8 to 8.64 µg/g, while 72% of *Aspergillus spp.* strains isolated from farm samples were identified as *A. flavus*, and 69,7% of them were able to produce 0.66 to 58.8 µg/g AFB₁.

Aflatoxin B₁ was detected in the three types of chinchilla feed from the farm. Lucerne cubes (feed C) showed the highest percentage of contaminated samples (55,5%), with levels between 1.97 and 40.40 µg/kg AFB₁. Contamination percentage of pelletized feeds A and B were 33,3 and 41,2%, respectively. Aflatoxin B₁ levels detected ranged from 3.84 to 97.34 µg/kg in feed A and 1.9 to 9.74 µg/kg in feed B. The mean AFB₁ levels in feed A and C exceeded the limit established by the Good Manufacturing Practices, which is 20 µg/kg (GMP 2008). Aflatoxin B₁ was not detected in any of the samples from the factory (Table 5).

The macroscopic examination of livers revealed they had a normal appearance, normal size, sharp borders and a reddish-brown color. Histopathology the livers indicated 63.3% of the organs showed slight to moderate lipid degeneration, with diffuse cytoplasm vacuolation, 9% showed intense lipid

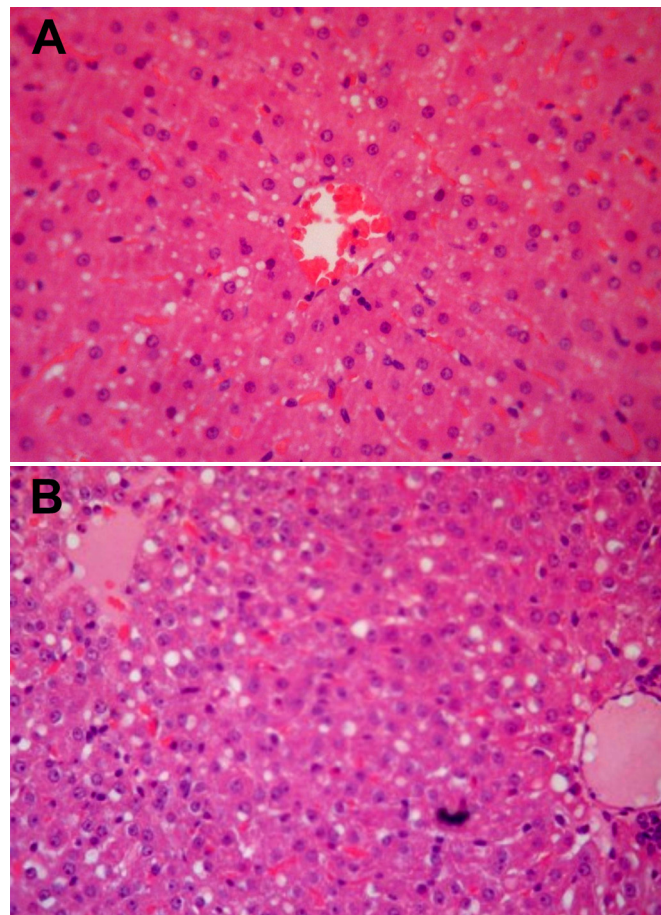


Figure 2. Livers of slaughtered chinchillas from the farm. a) Slightly diffuse cytoplasmic vacuolation of hepatocytes present in a section of a normal chinchilla liver. b) Moderated diffuse cytoplasmic vacuolation and necrosis localized in the periportal region. H.E. 400X.

cytoplasm vacuolation, while 27.3% showed hydropic degeneration and even nuclear vacuolation of hepatocytes from H.E. stained tissue sections (Figure 2).

DISCUSSION

The mycobiota, water activity, natural occurrence of AFB₁ in chinchilla feed samples and the aflatoxigenic capacity of *A. flavus* isolates obtained from

Table 5. Aflatoxins in chinchilla feeds.

Kind of feed ^a	AFB ₁ levels (µg kg ⁻¹)			
	Contaminated samples (%)	Samples exceeding permitted limit ^b	Range	Mean
Feed A	41.2	28.6	3.84- 97.34	24.76
Feed B	33.3	0	1.90- 9.74	5.66
Feed C	55.5	60	1.93- 40.4	23.64

^a Aflatoxin B₁ levels (µg kg⁻¹) and contamination frequency of different feeds (A: fur chinchillas, B: mother chinchillas and C: Lucerne cubes) sampled in a chinchilla farm.

^b 20 µg kg⁻¹ according to Good Manufacturing Practices (GMP, 2008).

these were evaluated. The macroscopic and microscopic characteristics and lesions in the livers of animals exposed to AFB₁ natural contamination levels were also studied.

In general, total fungal counts (CFU/g) on the three types of feed were moderate. Pelletized feeds did not exceed the feed hygienic quality limit (1×10^4 CFU/g) while lucerne cubes did slightly surpass this limit (GMP 2008). These results differ from studies that informed counts highly exceeding 1×10^4 CFU/g in different feeds intended for poultry, swine and cattle (Magnoli et al. 2002, Accensi et al. 2004, Rosa et al. 2006, Cavaglieri et al. 2009, González Pereyra et al. 2008a,b,c, 2009, 2011) and agree with others (Oliveira et al. 2006, Fraga et al. 2007) that reported lower counts in poultry feed samples. In our study, *Aspergillus* and *Fusarium* species showed the highest isolation frequencies, followed by *Penicillium* spp. Many studies have encountered species of these three genera as the dominant mycobiota in many animal feedstuffs (Bragulat et al. 1995, Magnoli et al. 2002, Richard et al. 2007, González Pereyra et al. 2008b,c, 2009, 2011, Ghiasian & Maghsood 2011). In our study, *A. flavus* was the prevalent species. This result concurs with many studies carried out by other authors who encountered this species in the highest frequency in cereals and different feeds and feed ingredients (Sanchis et al. 1993, Adebajo et al. 1994, Pitt et al. 1994, Pitt & Hocking 1997, Dalcero et al. 1997, Accensi et al. 2004, Saleemi et al. 2010). *Aspergillus* species belong to the fungal flora that typically appears during storage at low a_w in substrates such as grains and mixed feeds. The most frequently isolated species in this study have been described as moderately xerophilic (*A. flavus*) and slightly xerophilic fungi (*A. fumigatus*) (Lacey & Magan 1991). All samples showed *Fusarium* spp. contamination. As it has been informed for other animal feeds, *F. verticillioides* was the prevalent species (Oliveira et al. 2006, Campos et al. 2008).

A high percentage of *A. flavus* strains assayed were AFB₁ producers. Several authors have reported production of aflatoxins B and G by *A. flavus* isolated from maize, feeds and other substrates (El-Kady et al. 1994, Jan et al. 1995, Horn & Dorner 1999, Gatti et al. 2003, Saleemi et al. 2010).

The GMP (GMP, 2008) regulations on product standards in the animal feed sector established that the current maximum permitted level for AFB₁ for poultry feeds is 20 µg/kg. The mean AFB₁ levels of

feeds A and C collected from the farm slightly exceeded this limit, while feed B and the feeds from the factory did not. In some samples from the farm, AFB₁ levels were as high as 97 and 40 µg/kg for feeds A and C, respectively. Even though amounts of toxin detected on our chinchilla feeds were not enough to cause dramatically adverse effects in animals, such as an acute mycotoxicosis, it is a sign that the feed used in the farm was not of the best quality, and the levels of toxin detected could affect young animals (Jones et al. 1982). Furthermore, sublethal doses of mycotoxins produce a chronic toxicity that can result in liver cancer. Consumption of low doses of AFs for an extended period of time can cause reduction of the feed intake and feed conversion, weight loss and weak fur. The liver is the primary target organ of acute injury from AFs ingestion in all species. The diagnosis of mycotoxicoses is usually includes the analysis of the feed as well as the histopathology since clinical signs can be nonspecific and confusing. In a recent research, livers from 9 chinchillas that died naturally during the disease outbreak in a farm and livers from healthy chinchillas slaughtered for commercial pelt recovery were analyzed for their macroscopic and microscopic characteristics through necropsy and histopathology (González Pereyra et al. 2008a). Histopathologic analysis revealed hepatocellular changes typical of AFs intoxication such as cytoplasmic vacuolation, nodular hyperplasia, and bile-duct proliferation. Moreover, aflatoxins B₁, B₂, G₁ and G₂ levels high enough for causing an acute outbreak were detected in the feed consumed by the animals. In the present study, macroscopic inspection of the livers did not reveal the typical characteristics of acute toxicity such as general enlargement, yellowish coloration, hypertrophy, rounded hepatic borders or increased friability. Only slight histological changes that indicated hepatic toxicity (lipid vacuolation of hepatocytes) were observed in the microscopic analysis of H/E stained liver sections.

Analyses of the pelletized feed for AFB₁ by HPLC revealed that the feed samples were contaminated. The presence of AFB₁ producer species and the detection of this toxin (even in low levels) indicated that contamination in these kinds of feeds exists and constitutes a hazard for the animals, farmers and feed factory workers.

The current study revealed that toxigenic fungal species can contaminate feed intended for chinchillas in breeding farms. Toxigenic strains of *A.*

flavus able to produce AFB₁ as well as the toxin itself were detected in feeds inducing moderate changes in the animals liver histopathology. This fact suggests that periodic monitoring of the feeds and their ingredients would be required in order to prevent acute outbreaks and economic losses caused by chronic AFs exposure.

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