

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

EXPOSICIÓN CRÓNICA A HONGOS PRODUCTORES DE AFLATOXINAS RELACIONADA A DAÑOS HEPÁTICOS EN CHINCHILLAS (*Chinchilla lanigera*) DESTINADAS A LA PRODUCCIÓN DE PIEL

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Abstract

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 chinchillas, B: mother chinchillas, 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⁻¹. *Aspergillus*, *Fusarium* and *Penicillium* were the prevalent genera, while *A. flavus*, *A. fumigatus*, *F. verticillioides* and *F. proliferatum* were the prevalent species. 50 %

of *A. flavus* strains from factory samples and 69.7 % from farm samples produced 2.78 to 8.64 µg g⁻¹ and 0.66 to 58.8 µg g⁻¹ 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⁻¹ AFB₁. Mean levels in feed A and C exceeded 20 µg kg⁻¹. 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.

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Key words: Aflatoxins, *Aspergillus* spp., Chinchilla lanigera, feed, histopathology, toxicology.

Resumen

La piel de chinchilla es una de las más exóticas y apreciadas en el mercado internacional. La cría de estos animales es una actividad muy rentable. En Argentina, se han detectado casos de aflatoxicosis aguda en criaderos. Los objetivos de este trabajo fueron: i) estudiar la microbiota y los hongos productores de aflatoxina B₁ (AFB₁) presentes en alimento para chinchillas. ii) analizar la contaminación natural con AFB₁ de estos alimentos iii) buscar lesiones histopatológicas en hígados de chinchillas de los criaderos. Se recolectaron muestras de diferentes alimentos (A: chinchilla piel, B: chinchilla madre, C: cubos de alfalfa) en una fábrica y un criadero localizados en la ciudad de Río Cuarto, en la región central de Argentina. Los hígados de las chinchillas sacrificadas en el criadero fueron analizados macroscópica y microscópicamente. Los recuentos fúngicos totales fueron mayores a 1x10⁴ UFC g⁻¹. *Aspergillus*, *Fusarium* y *Penicillium* fueron los géneros prevalentes, mientras que *A. flavus*, *A. fumigatus*, *F. verticillioides* y *F. proliferatum* fueron las especies aisladas con mayor frecuencia. 50 % de las cepas de *A. flavus* aisladas de la fábrica y 69.7 % de las aisladas del criadero produjeron 2.78 a 8.64 µg g⁻¹ y 0.66 a 58.8 µg g⁻¹ de AFB₁, respectivamente. Se detectó AFB₁ sólo en las muestras del criadero, con mayor incidencia en el alimento C. Los niveles de toxina variaron entre 1.90 y 97.34 µg kg⁻¹. Los niveles promedios en A y C fueron superiores a 20 µg kg⁻¹. El análisis macroscópico de los hígados reveló apariencia, tamaño y color normal. El análisis microscópico indicó que 63.3 % de los hígados presentaron degeneración lipídica leve a moderada con vacuolización difusa del citoplasma, 9 % presentaron vacuolización lipídica intensa y 27.3 % degeneración hidrópica y vacuolización nuclear en los hepatocitos. El monitoreo periódico de la calidad de los alimentos e ingredientes usados en la alimentación de chinchillas puede evitar

intoxicaciones agudas y pérdidas económicas causadas por la exposición crónica a aflatoxinas.

Palabras clave: Aflatoxinas, *Aspergillus* spp., Chinchilla lanigera, histopatología, toxicología.

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 and Hagler, 2002; Monbaliu *et al.*, 2010).

Aflatoxins (AFs) are secondary metabolites produced by toxigenic strains of *A. flavus* and *A. parasiticus*, mainly. Chemically, AFs belong to the group of bifuran coumarins, being aflatoxins B₁ (AFB₁), the most toxic and hazardous one. Aflatoxin B₁ is hepatotoxic, highly mutagenic, carcinogenic and probably teratogenic to animals (Smith and 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 non-reversible detoxification via the formation of hydroxylated metabolites (AFM₁, AFQ₁, AFP₁, AFB_{2a}), 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 of the Andes Mountains in South America. At the pre-

sent 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 AFB₁ 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 chinchilla 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 12 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.*, 1994; ISO 21527-1) and dichloran 18 % glycerol agar (Pitt and 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^{-1}). On the last day of incubation, individual CFU g^{-1} 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 and 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. Fifty 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 N_2 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 AFB_1 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 AFB_1 through visual comparison with the standard solution of known concentration. Limit of detection (LOD) of this method was 5 $\mu\text{g kg}^{-1}$.

Aflatoxin analysis of feed samples

Aflatoxin B_1 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 AflaPat 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 N_2 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^{-1} flow rate. Fluorescence of aflatoxin derivatives was recorded at 360 nm excitation 460 nm emission wavelengths. Standard curves were constructed using AFB_1 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^{-1} .

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 analyses

Analysis of CFU g^{-1} was performed by Mixed and General Lineal Model. Fisher's LSD test was done to compare means of treatments (Quinn and 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^{-1}) of chinchilla feeds varied from 1×10^2 to 1.4×10^6 (Table 1). Total fungal counts of lucerne cubes exceeded the limit established for good quality feeds and feed ingredients by the Good Manufacturing Practices (1×10^4 CFU g^{-1}) (GMP, 2008).

Table 1.
Fungal counts in chinchilla feeds.
Total fungal counts (UFC g⁻¹)

Source of Samples	Kind of sample	DRBC	Contaminated samples exceeding recommended limits (%) [*]	
			DG18	
Farm	feed A	1.0 x10 ² - 1 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

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.

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

Table 2.
Statistical analysis of fungal counts in chinchilla feeds.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<i>Source of sample</i>	1	2.45	2.45	1.30	0.2540
<i>Kind of feed</i>	2	297.77	148.89	79.31	<0.0001
<i>Media</i>	1	0.35	0.35	0.19	0.6646
<i>Source: Kind of feed</i>	1	4.74	4.74	2.53	0.1128
<i>Kind of feed: Media</i>	2	8.10	4.05	2.16	0.1168
<i>Source: Kind of feed:Media</i>	1	0.11	0.11	0.06	0.8114
<i>Residuals</i>	416	780.89	1.88		

Analysis of CFU g⁻¹ by Mixed and General Lineal Model. Software: PROC GLM in SAS (SAS Institute, Cary, NC, USA).

There was 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) (Table 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 fre-

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

Kind of feed	Mean	SE*
A	1.67 ^b	0.13
B	2.01 ^b	0.16
C	4.82 ^a	0.29

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

* Standard error.

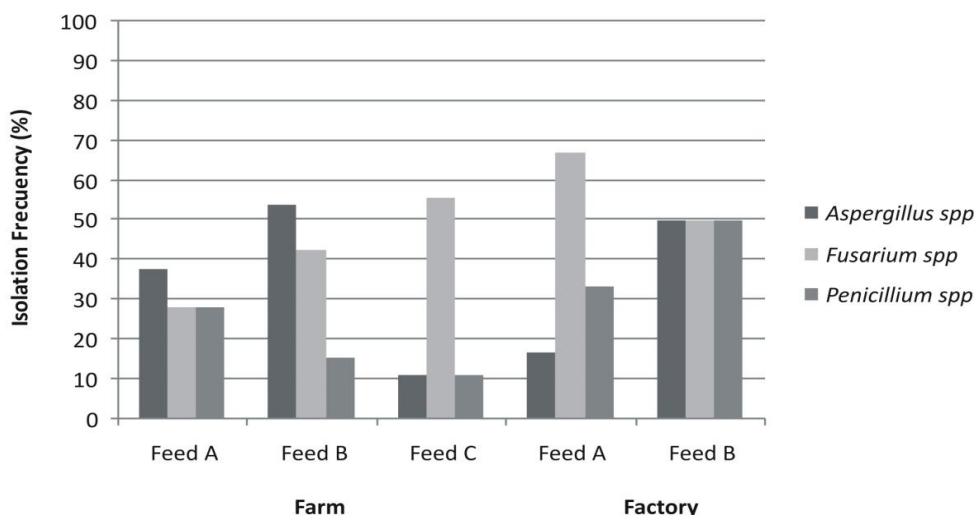


Figure 1. Mycobiota of chinchilla feeds. Isolation frequency (%) of fungal genera identified in different kinds of chinchilla feeds (A: fur chinchillas, B: mother chinchillas and C: Lucerne cubes) from different sources (farm and factory).

quent genera found was *Fusarium* followed by *Penicillium* and *Aspergillus* (Figure 1).

Aspergillus and *Fusarium* species were identified. *Aspergillus flavus*, *A. fumigatus*, *F. verticillioides* 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⁻¹, 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⁻¹ AFB₁.

Table 4.
***Aspergillus* and *Fusarium* species in chinchilla feeds.**
Fungal species relative density (%)

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						

Distribution (%) 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).

Table 5.
Aflatoxins in chinchilla feeds.

Kind of feed	AFB ₁ levels (µg kg ⁻¹)			
	Contaminated samples (%)	Samples exceeding permitted limit*	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

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

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⁻¹ 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⁻¹ in feed A and 1.9 to 9.74 µg kg⁻¹ 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⁻¹ (GMP, 2008)(Table 5). Aflatoxin B₁ was not detected in any of the samples from the factory.

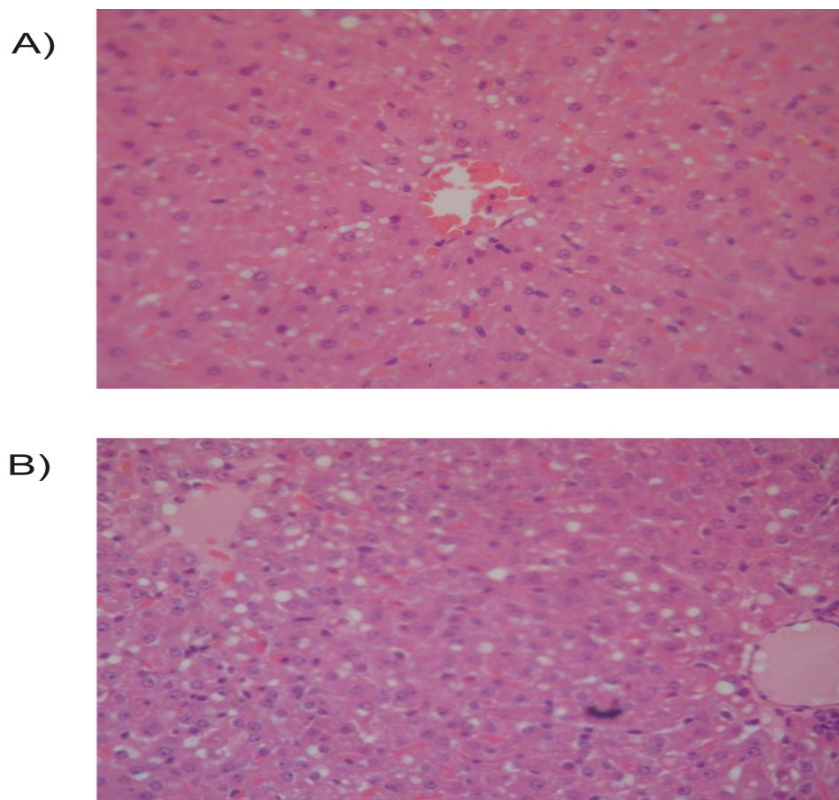


Figure 2. Histopathology of chinchilla livers. Histopathology of liver sections from slaughtered chinchillas from the farm stained with Hematoxylin and Eosin (H/E). A) Slightly diffuse cytoplasmic vacuolation of hepatocytes present in a section of a normal chinchilla liver. B) Moderated diffuse cytoplasmic vacuolation and necrosis localized in the periportal region.

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 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 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⁻¹) on the three types of feed were moderate. Pelletized feeds did not exceed the feed hygienic quality limit (1x10⁴ CFU g⁻¹) while lucerne cubes did slightly surpassed this limit (GMP, 2008). These results differ from studies that informed counts highly exceeding 1x10⁴ CFU g⁻¹ 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; González Pereyra *et al.*, 2008b; González Pereyra *et al.*, 2008c; González Pereyra *et al.*, 2009; González Pereyra *et al.*, 2011) and

agree with others (Fraga *et al.*, 2007; Oliveira *et al.*, 2006) 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 (Magnoli *et al.*, 2002; González Pereyra *et al.*, 2008b; González Pereyra *et al.*, 2008c; González Pereyra *et al.*, 2009; González Pereyra *et al.*, 2011; Bragulat *et al.*, 1995; Richard *et al.*, 2007; Ghiasian and Maghsoud, 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 (Pitt and Hocking, 1997; Accensi *et al.*, 2004; Adebajo *et al.*, 1994; Dalcerro *et al.*, 1997; Sanchis *et al.*, 1993; Pitt *et al.*, 1994; 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 and 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 the *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 (Saleemi *et al.*, 2010; El-Kady *et al.*, 1994; Jan *et al.*, 1995; Horn and Dorner, 1999; Gatti *et al.*, 2003).

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⁻¹. 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⁻¹ 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 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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