Research Article

Mycoflora and Natural Incidence of Selected Mycotoxins in Rabbit and Chinchilla Feeds

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Mycotoxins are secondary metabolites produced by filamentous fungi that cause a toxic response when ingested by animals or man. Demand of natural fur, such as those from rabbit and chinchilla, produced under controlled conditions, has increased worldwide. The toxicogenic mycoflora contaminating feeds for these animals was enumerated and identified. Six of the major mycotoxins implicated in animal mycotoxicosis were detected and quantified. Moulds count ranged from >10 to 4.7 × 10^5 CFU g^-1; 14% of the samples exceeded the limit that determines hygienic feed quality. More than twenty species belonging to the five most important mycotoxigenic mould genera were recovered. Among the analyzed mycotoxins, aflatoxins were recovered in 100% of the examined samples, deoxynivalenol in 95%, fumonisins in 100%, ochratoxin A in 98%, T2 toxin in 98%, and zearalenone in 100%. Cooccurrence of mycotoxins was observed in 100% of the samples analyzed. Exposure to multiple mycotoxins was thus demonstrated for these animals.

1. Introduction

There is an increasing worldwide demand of natural fur produced under controlled conditions. Among them, chinchilla, fox, mink, and otter fur are quite appreciated. On the other hand, rabbit breeding besides fur provides meat intended for human consumption [1]. Worldwide production of rabbit meat was 1.1 million tons per year between 2002 and 2005 and has grown around 49% over the last 15 years with China, Italy, Spain, and France being the main producers [2]. In America, Argentina is the main producer and exporter. In 2004 Argentina exported 1400 tons of rabbit meat to the EU [2].

Commercial feedstuffs are the main consumables in rabbit and chinchilla breeding representing 60–70% of production cost [3]. Filamentous fungi are ubiquitous in nature and responsible for producing mycotoxins in agricultural crops [4]. Rabbit and chinchilla feed ingredients that constitute complete feed products are derived from different raw materials. Inadequate management of raw materials can lead to undesirable growth of fungi, leading to loss of nutritive substances and resulting in contamination by mycotoxins [5]. Lowered production, illness, and death can be consequences of mycotoxin-contaminated feeds [6].

One of the main features of mycotoxicosis in rabbits is feeding reduction (about 20–60%) which causes a delay in growth and thus reduced productivity. Mycotoxicosis in rabbits includes acute or chronic pathologies depending on the mycotoxin involved, its concentration, period of exposure, accumulative effects, and synergisms among mycotoxins. In some cases abortion and death of adults may occur [7]. Also, it is necessary to consider that even though there
is little information available regarding the prevalence and concentration of mycotoxins in foods of animal origin, some toxins ingested by animals may be found in meat, milk, or eggs [8]. Regular monitoring of toxigenic mycoflora of the agriculturally based feeds and foods is an essential prerequisite for development of strategies to control or prevent mycotoxin exposure of animal and human populations [9].

Despite the great attention that has been paid to the study of toxicogenic moulds and their mycotoxins in various feeds, little is known about fungal and mycotoxin contamination of mixed chinchilla and rabbit feed. Thus, the aim of this work was to study the toxicogenic mycoflora including enumeration and identification of mould genera and species naturally contaminating feeds for these animals along with detection and quantification of the major mycotoxins: aflatoxins, ochratoxin A, T-2 toxin, fumonisins, deoxynivalenol (DON), and zearalenone.

### 2. Materials and Methods

#### 2.1. Sample Collection and Preparation

A total of 42 representative samples (1-2 kg per sample) of finished rabbit (17) and chinchilla (25) feeds were collected from 7 companies in 5 provinces of Argentina (Buenos Aires, Córdoba, La Pampa, La Rioja, and Mendoza) in 2010. All samples were homogenized and divided to obtain a 1 kg working sample for analysis. Each sample was ground in a laboratory mill. For mycological examination feed samples were immediately analyzed upon arrival or they were stored for 2-3 days in paper bags at room temperature (about 25°C). Feed samples intended for mycotoxin analysis were stored at −20°C.

#### 2.2. Mycological Analysis

The dilute plate technique was used for enumeration and isolation of fungi [11]. Ten grams of each milled feed sample was mixed with 90 mL of 0.1%
peptone and shaken on a horizontal shaker for 20 minutes. Then, 0.1 mL of a proper spore suspension dilution (made up to 105 spores per mL) was inoculated onto the following media: dichloran rose bengal chloramphenicol agar (DRBC) to enumerate total culturable fungi, dichloran 18% glycerol agar (DG18) to enumerate xerophilic fungi, and dichloran chloramphenicol peptone agar (DCPA) for selective isolation of Alternaria and Fusarium species [11]. Plates were incubated at 25°C for 7 days. The DCPA plates were incubated under a 12 h of light: 12 h of darkness photoperiod. For counting, plates containing 10–100 colonies were used and the results were expressed as colony-forming units per gram of sample (CFU g−1) [11]. Individual CFU g−1 counts for each colony type, considered to be different, were recorded. Representative colonies of each type were transferred for sub-culturing onto plates with malt extract agar (MEA) or water agar (WT), for moulds suspected to belong to Alternaria or Fusarium genera. Filamentous fungi were identified at genus level according to macro- and microscopic criteria in accordance with Samson et al. [12]. Fungal isolates were identified at species level according to the leading authorities: Penicillium and Aspergillus spp. according to Pitt and Hocking [11], Fusarium spp. according to Nelson et al. [13], Alternaria spp. according to Simmons [14], and other fungi according to Pitt and Hocking [11]. The isolation frequency (Fr) and relative density (RD) of genus/species were calculated according to González et al. [15], Pacin et al. [16], and Salemi et al. [9] as follows:

Fr (%) = number of samples with a genus or species/total number of samples × 100.

RD (%) = number of isolates of a genus or species/total number of fungi isolated × 100.

All the isolates were preserved on agar slants of malt extract agar (MEA) or potato dextrose agar (PDA) for Alternaria and Fusarium at 4°C and cryopreserved in 18% glycerol at −20°C.

2.3. Mycotoxin Analysis. To evaluate mycotoxin occurrence, feed samples were subjected to quantitative analyses using ELISA-based analytical test kits for aflatoxins, ochratoxin A, T-2 toxin, fumonisins, deoxynivalenol (DON), and zearalenone (RIDASCREEN FAST, R-Biopharm AG). The extraction procedures were according to manufacturer protocols. In brief, 5 g of ground sample was extracted with 25 mL of 70% methanol for aflatoxins, T-2 toxin, zearalenone, and fumonisins. For ochratoxin A and DON, samples were extracted with 12.5 mL of 70% methanol or 100 mL of distilled water, respectively. Afterwards, samples were shaken vigorously for 3 minutes and the extracts filtered through the Whatman N°1 paper. Then, aflatoxin, ochratoxin A, T-2 toxin, and zearalenone filtrates were diluted with distilled water in the ratio 1:1 and fumonisin filtrates in the ratio 1:4. Fifty μL of the diluted filtrate per well were used for testing.

3. Results

This study shows that fungi and mycotoxins were present in all the feed samples assayed. Fungal counts (CFU g−1) on each medium are shown in Table 1. Total fungal counts on DRBC ranged from <10 to 4.7 × 105 CFU g−1. High fungal contamination was found in 6 out of 42 samples, exceeding the limit of 1 × 104 CFU g−1, that determines feed hygienic quality [17]. Xerophile moulds counts ranged from <10 to 8.5 × 105 CFU g−1. Spoilage of feedstuff can be due to xerophilic fungi, which are capable of rapid growth above about 0.77 a w and of slow growth at 0.75 a w and below, downto about 0.68 a w [11]. On DCPA fungal counts ranged from <10 to 2.7 × 105 CFU g−1.

Both field and storage fungi were found in this study. Mycotoxigenic genera such as Aspergillus, Penicillium, Fusarium, and Alternaria were recorded. These moulds are of great importance due to potential mycotoxin production, which can contaminate many agricultural commodities like wheat, oat, barley, sunflower, soybean, and so forth, used in the formulation of finished feeds. Cladosporium, Trichoderma, and other mitosporic Ascomycetes were also found. One genus belonging to mycotoxigenic Ascomycetes, Eurotium, and one genus belonging to Zygomycetes, Mucor, were determined. The most frequent fungi were those from the genus Eurotium, recovered from 29 samples (Fr 69%). In the second place were moulds from the genus Aspergillus, recovered from 22 samples (Fr 52.4%). moulds from the genus Cladosporium (Fr 42.5%), Penicillium (Fr 33.3%), and Mucor ( Fr 33.3%) and yeast (Fr 40.5%) were recovered with relative high frequency. In less proportion, other genera recovered were Fusarium, Alternaria, Trichoderma, Scopulariopsis, and Paecilomyces (Table 2).

The fungal species isolated on different agar media are shown in Table 3. This table also illustrates the frequency

| Parameter | Aflatoxins | Deoxynivalenol | Mycotoxins (ppb) |
|-----------|------------|----------------|------------------|
| No. of samples tested | 42 | 42 | 42 |
| No. of positive samples | 42 | 40 | 42 |
| Percentage positive (%) | 100% | 95% | 100% |
| Range (ppb) | <1.70–22.55 | 222–1740 | 222–6000 |
| Median (ppb) | 7.26 | 743 | 462 |
| Highest level (ppb) | 22.55 | 1740 | 6000 |

Table 4: Number of samples tested, number of positive samples, and percentage and levels of detected mycotoxins.
Table 5: Concentration of different mycotoxins in chinchilla and rabbit feed samples. ND: not detected; CH: chinchilla; R: rabbit.

| Samples tested | Aflatoxins (ppb) | Deoxynivalenol | Fumonisins | Ochratoxin A | T2 toxin | Zearalenone |
|----------------|------------------|----------------|------------|--------------|-----------|-------------|
| CH1            | 3.91             | 393            | 222        | 5            | 50        | 50          |
| CH2            | 1.84             | ND             | 222        | 8.09         | 50        | 50          |
| CH3            | 1.7              | ND             | 222        | 9.74         | ND        | 50          |
| CH4            | 6                | 737            | 222        | 7.77         | 50        | 50          |
| CH5            | 6.08             | 761            | 331        | 7.83         | 50        | 50          |
| CH6            | 7.22             | 1592           | 222        | 6.24         | 50        | 50          |
| CH7            | 7.19             | 222            | 270        | 6.53         | 50        | 50          |
| CH8            | 5.73             | 610            | 795        | 12.48        | 50        | 50          |
| CH9            | 5.55             | 1080           | 598        | 10.17        | 50        | 80.17       |
| CH10           | 4.26             | 1210           | 370        | 8.95         | 50        | 50          |
| CH11           | 6.24             | 868            | 525        | 8.66         | 50        | 50          |
| CH12           | 7.64             | 629            | 732        | 8.6          | 50        | 50          |
| CH13           | 8.6              | 488            | 503        | 13.19        | 50        | 50          |
| CH14           | 8.77             | 409            | 370        | 10.23        | 50        | 50          |
| CH15           | 9.24             | 760            | 866        | 23.49        | 50        | 82.12       |
| CH16           | 7.09             | 1210           | 222        | 23.49        | 50        | 68.69       |
| CH17           | 6.92             | 1300           | 222        | 15.25        | 50        | 75.59       |
| CH18           | 7.5              | 548            | 626        | 12.63        | 50        | 53.96       |
| CH19           | 8.77             | 937            | 1030       | 11.81        | 50        | 50          |
| CH20           | 8.24             | 798            | 715        | 23.34        | 50        | 50          |
| CH21           | 10.17            | 1720           | 222        | 25.38        | 50        | 58.34       |
| CH22           | 9.46             | 241            | 447        | 11.59        | 50        | 66.49       |
| CH23           | 6.67             | 1390           | 975        | 16.91        | 50        | 79.9        |
| CH24           | 10.98            | 1660           | 498        | 11.59        | 50        | 78.81       |
| CH25           | 9.15             | 462            | 340        | 10.9         | 50        | 71.2        |
| R1             | 22.55            | 327            | 6000       | 5            | 50        | 50          |
| R2             | 3.66             | 222            | 236        | ND           | 81.75     | 50          |
| R3             | 1.7              | 355            | 222        | 5            | 50        | 50          |
| R4             | 1.99             | 279            | 222        | 5            | 50        | 50          |
| R5             | 7.2              | 222            | 3110       | 6.6          | 50        | 50          |
| R6             | 5.57             | 256            | 1500       | 5.76         | 50        | 50          |
| R7             | 7.46             | 222            | 376        | 5.55         | 50        | 50          |
| R8             | 9.72             | 916            | 562        | 5            | 50        | 50          |
| R9             | 9.93             | 1300           | 953        | 17.44        | 50        | 50          |
| R10            | 9.97             | 1100           | 581        | 26.57        | 50        | 50          |
| R11            | 7.96             | 700            | 709        | 8.43         | 129.88    | 50          |
| R12            | 6.14             | 1190           | 477        | 8.54         | 120.27    | 50          |
| R13            | 7.14             | 749            | 241        | 16.81        | 128.98    | 50          |
| R14            | 9.6              | 222            | 786        | 12.18        | 50        | 177.97      |
| R15            | 7.94             | 1740           | 311        | 13.11        | 50        | 140.38      |
| R16            | 9.19             | 1690           | 390        | 9.54         | 50        | 105.36      |
| R17            | 7.29             | 467            | 492        | 7.01         | 50        | 50          |

and relative density of recovered species. Among Eurotium species, E. amstelodami was the most prevalent (Fr 50%). Other Eurotium spp. recovered were E. chevalieri, E. repens, and E. rubrum. Mycotoxigenic species such as A. flavus, A. parasiticus, P. expansum, P. roqueforti, F. proliferatum, and F. subglutinans were also found in our work. There is only limited data on the occurrence of important mycotoxins in rabbit feed; thus, research and surveys in this
area are very important [10]. In our study we have determined that all samples were contaminated with mycotoxins (Table 4). The concentration of different mycotoxins in finished mixed chinchilla and rabbit feed samples is shown in Table 5. Recommended maximum amount of mycotoxins in rabbit feed is shown in Table 6 [10]. Though only one sample had aflatoxin and fumonisins concentrations exceeding the recommended limits, several toxins have been detected in low concentrations in all the samples, which might lead to a response of synergic toxicity in animals under this type of exposure (Table 5).

4. Discussion

Fungal growth on raw materials used as ingredients (in the field or during silage storage) leads to contamination of the final feed. This fungal growth reduces nutritional value and may result in the production of mycotoxins, which constitute a risk factor for animal health [5, 18]. Mycotoxigenic fungi, such as those we found in our work, are undesirable because of their potential for mycotoxin production. Other fungi isolated such as 

| Mycotoxin          | Maximum content for feed with a moisture content of 12% |
|--------------------|---------------------------------------------------------|
|                    | ppm (mg/kg) | ppb (µg/kg) |
| Aflatoxin B1       | 0.02        | 20          |
| Ochratoxin A       | 5           | 5000        |
| Deoxynivalenol     | 5           | 5000        |
| Zearalenone        | 0.50        | 500         |
| Fumonisins B1 + B2 | 5           | 5000        |

Although the scientific literature offers a broad variety of information about the effects of individual mycotoxins on various animal species, concurrent exposure to multiple mycotoxins is more likely in the livestock industry. Poor livestock performance and/or disease symptoms may be due to the synergistic interactions between multiple mycotoxins [28]. In our work, cooccurrence of mycotoxins was demonstrated in 100% of the samples assayed (Tables 4 and 5). In Argentina, there is rather limited information concerning natural occurrence of mycotoxins in feedstuffs, particularly with respect to rabbit feed. Only one work reported that 25% of rabbit feed samples from Córdoba province were contaminated with ochratoxin A with a mean level of 21.8 ppb [29]. Also there is scare information from other parts of the world. Mohanamba et al. [30] reported that 77% of rabbit feed samples were contaminated with aflatoxins in India.

5. Conclusions

The present study has provided information about the contaminating toxigenic mycoflora in rabbit and chinchilla feeds in Argentina. This is the first report describing the cooccurrence of six mycotoxins. These toxic substances are known to be either carcinogenic, neurotoxic, nephrotoxic, dermatotoxic, or immunosuppressive. Although the synergic effects of mycotoxins on health and productivity of other animal species such as poultry have been well documented [31], more studies are needed in order to screen the presence of different mycotoxins in different feeds. Particular attention should be paid to the cooccurrence and synergic effects of mycotoxins present in low levels in order to avoid the consumption of contaminated feeds which could provoke acute or chronic illnesses leading to economic losses.

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