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The occurrence of aflatoxigenic *Aspergillus* spp. in dairy cattle feed in Southern Brazil

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ABSTRACT

The presence of mycotoxins or related fungi in animal feed is a major problem for animal and human health. Silage and concentrated feed samples were collected from 21 dairy farms in the Western part of Paraná state in Southern Brazil. Water activity and pH of all samples were measured, and each sample was analyzed to check for the presence of aflatoxigenic *Aspergillus*. Water activity was observed to be lower in the concentrated feed samples. The pH was lower in the silage samples, indicating fermentation processes. Two silage samples and four concentrated feed samples were contaminated with *Aspergillus* spp. Seven isolates of *Aspergillus* spp. were obtained and their potential to produce aflatoxins was evaluated. Four of the isolates, two from the silage samples and two from the concentrated feed samples, produced the aflatoxins B1, B2, G1, and G2 in culture media. These isolates were identified as *Aspergillus parasiticus* and *Aspergillus nomius*. The presence of aflatoxigenic isolates of *Aspergillus* spp. in silage and concentrated feed samples is a matter of concern, because of the risk of aflatoxin production and contamination of the animal feed.

Introduction

Various species of the genus *Aspergillus* are commonly isolated from stored foods.[1,2] Aflatoxins are mycotoxins produced by the species of the genus *Aspergillus*, subgenus *Circumdati* section *Flavi* (also referred to as the *Aspergillus flavus* group) mainly by the species *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*.[3–6] About 50% of the isolates of the species *A. flavus* predominantly produce aflatoxins B1 and B2. Nearly all the isolates of the species *A. parasiticus* produce aflatoxins B1, B2, G1, and G2.[3,4] Aflatoxin M1, which is secreted in milk from the mammary glands of both humans and lactating animals, is a hydroxylated metabolite of aflatoxin B1.[7]

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Approximately 0.5–6% of the ingested aflatoxin B1 is converted to aflatoxin M1 and is secreted in milk.[7] According to the International Agency for Research on Cancer (IARC), aflatoxins are classified as group I or carcinogenic to humans, being aflatoxin B1 the most toxic.[8] Aflatoxin M1 is as toxic as aflatoxin B1 but is ten times less carcinogenic. The susceptibility to aflatoxins depends on the species, age, dose, the extent of exposure, nutrition, gender, and concomitant exposure to other toxins. The liver is the primary target organ in mammals, and aflatoxins cause hepatocellular carcinoma.[3,4]

Milk has high nutritive value because it contains many macro- and micronutrients, which are important for the growth of children and maintenance of human health. Aflatoxin M1 is thermostable and resistant to pasteurization.[7] Humans can be exposed to aflatoxin M1 through endogenous production or by intake of dairy products. Babies and young children, who might consume contaminated milk or be exposed by breastfeeding, are the most vulnerable.[7]

According to the United States Department of Agriculture, Brazil was the sixth largest milk producer in the world in 2015, only behind European Union, United States, India, China, and Russia, achieving the production of 35 billion liters.[9] According to the Brazilian Institute of Geography and Statistics, there has been an increase in the milk production by more than 50% during the past few years, as compared to the beginning of the 21st century.[10] The highest increase has occurred in the Southern region, which is the major milk producing region in Brazil, contributing to 35.2% of the national production in 2015. In addition, this region shows a productivity of 2900 L/cow/year, which is 80% higher than the Brazilian average of 1609 L/cow/year.[10]

Milk productivity is related to the animal's productive potential and breed genetics.[11] The productive potential is favored by environmental factors such as climatic conditions and adequate nutrition. To compensate for poor growth of pastures, several dairy farms use forages, concentrates, and preserved feeds (hay or silage). As the production is intensified, these supplements become the sole source of animal feed. This is where the aflatoxins become a matter of concern.[12–14]

Because of a strong correlation between the presence of mold and the occurrence of mycotoxin, it is important to search for the presence of fungi in animal feed.[15–17] This information can indicate the Hazard Analysis Critical Control Point (HACCP) within the food production chain.[18] The objective of this study was to analyze animal feed for the presence of aflatoxigenic *Aspergillus* in dairy farms located in the western region of the Paraná State in Southern Brazil.

Materials and methods

Sample collection

Twenty-one randomly chosen dairy farms were visited during January of 2015. The dairy farms were located in the city of Marechal Cândido Rondon, in the Western region of the Paraná State in Southern Brazil. Before the sample collection, a questionnaire was answered by the farmers to identify what type of feed are provided for the animals, how they are fed, and how the feed is stored. The silages were well compacted and had the characteristic color and odor of optimum lactic acid fermentation. The outer layers of silage that were in contact with air (without the polystyrene cover) appeared dry, and some parts showed slight fungal contamination (spot). Each silage sample consisted of three sub-samples, categorized as Surface – composite sample obtained from the silo front; Depth – composite sample obtained from the silo interior at 25 cm depth; and Spot – composite sample obtained near contaminated points, within a radius of up to 20 cm, without collecting visibly degraded or contaminated material.[19]

The samples of the concentrated feed were also collected from most of the farms. Several storage forms of the concentrated feed were identified in the visited farms. For instance, bulk silos, feed bags purchased directly from agricultural holdings, and ingredients purchased in bulk and stored in reused bags or compartments within the farm itself. When the concentrated feed was stored as a silo, a sample was collected from the exit point of the silo. When the concentrated feed was kept in bags, samples were collected from various points of the bag in case of a single bag being used, or from several bags if more than one bag were being used. Each sub-sample consisted of several small samples, which were homogenized individually.

Measurement of water activity and pH of silage and concentrated feed samples

The measurement of water activity of the silage samples was performed using a LabSwift water activity instrument (Novasina, Lachen, Switzerland). The sample preparation and operation of the apparatus was performed according to the instructions described in the operating manual. After homogenizing the sample, a portion was transferred to and packed in a test dish in triplicate.

The pH measurements of the silage samples were performed by adding 9 g of the silage to 60 mL of water in a 250-mL beaker. After mixing for 5 min, the samples were left to rest for 30 min and an aliquot from the supernatant was used to measure the pH using a pH meter calibrated with standard buffer solutions of pH 4.0 and 7.0.[20]

Isolation of the microorganisms

Twenty grams of each silage or concentrated feed samples were added to 80 mL of sterile 0.1% peptone water in 250 mL Erlenmeyer flasks. This suspension was incubated at 25 °C for 1 h with agitation at 100 rpm. Aliquots of 100 μL of this suspension were spread on the surface of a Petri dish of diameter 10 cm (in triplicate) containing *A. flavus* and *parasiticus* agar (AFPA) (2 g/dL yeast extract; 1 g/dL bacteriological peptone; 0.05 g/dL ferric ammonium citrate; and 1.5 g/dL agar).[2] Rapidly growing molds such as *Rhizopus* and *Mucor* were inhibited by the addition of malachite green at a concentration of 2.5 μg/mL to the medium, before autoclaving. To prevent bacterial growth, 641 IU/mL of penicillin and 256.4 μg/mL of streptomycin were aseptically supplemented to the medium, after autoclaving and cooling to 60 °C. The culture was incubated at 25 °C with a photoperiod of 12 h for five days.

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