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Aflatoxins occurrence through the food chain in Costa Rica: Applying the One Health approach to mycotoxin surveillance

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ABSTRACT

Aflatoxins (AFs) are toxic metabolites produced by *Aspergillus* spp. and commonly found in crops, grains, feedstuff, and forages. Exposure to AFs has been associated with increased risk of liver cancer and growth retardation in humans, liver damage, immunosuppression, embryotoxicity in both animals and humans, and decreased milk, egg and meat production in animals. For the first time, the Costa Rican national mycotoxin surveillance programs for animal feed and food are considered as a whole, applying the One Health approach to the mycotoxin epidemiological research. Therefore, the aim of this study was to determine the occurrence of AFs in cereals, nuts, grains intended for animal and human consumption in Costa Rica.

In animal feed and feed ingredients, 970 samples were analyzed for AFs from 2010 to 2016 with an overall prevalence of positive samples of 24.0% (ranging from 0.01 to 290 μg kg^{-1}). Only 2.5% of the samples failed to comply the regulation for total AFs (20 μg kg^{-1} feed). From 5493 samples of agricultural commodities intended for human consumption analyzed from 2003 to 2015, there was an overall prevalence of AF positive samples of 10.8% (ranging from 0.48 to 500 μg kg^{-1}), and 2.8% did not comply the regulation for AFs (20 μg kg^{-1}). In both feed and food, the highest AF prevalence corresponded to corn ingredients (27.8%) and white corn (38.6%), respectively. Among the commodities intended for human consumption, red beans had the highest aflatoxin concentrations (500 μg kg^{-1}).

1. Introduction

Mycotoxins are secondary metabolites produced by fungi, mainly saprophytic, that can affect crops in the field, during harvest, and storage. Aflatoxins (AFs) are mycotoxins classified as furanocoumarins, produced by *Aspergillus flavus* and *A. parasiticus* (CAST, 2003). *Aspergillus flavus* has a worldwide distribution and produces aflatoxin B_1 (AFB_1) and aflatoxin B_2 (AFB_2). *Aspergillus parasiticus* produces aflatoxins B_1, B_2, G_1 (AFG_1) and G_2 (AFG_2). Aflatoxins have been classified as human carcinogens (De Ruyck, De Boevre, Huybrechts, & De Saeger, 2015; IARC, 2015), associated with children stunting (Wu, 2013), hepatotoxic for animals and humans (Hgindu, Johnson, & Kenya, 1982), genotoxic, immunotoxic, and responsible for growth retardation and decreased production in animals (Coulombe, Guarisco, Klein, & Hall, 2005; Grace, 2013; Stoev, 2015). Within the aflatoxin group, AFB_1 is the furthermost fraction found in food and it has the highest genotoxic and carcinogenic potential (De Ruyck et al., 2015). Furthermore, aflatoxin M_1 (AFM_1), the primary monohydroxylated derivative of AFB_1, may be present in milk from animals exposed to AFB_1 contaminated feed (EFSA, 2007; Marín, Ramos, Cano-Sancho, & Sanchis, 2013).

Incidence of *Aspergillus* infection and the concomitant contamination with AFs can occur in a wide variety of products and byproducts intended for animal and human consumption (Stoev, 2015). Such ingredients include corn, rice, peanut, sorghum, wheat, and soybean. Additional feed ingredients commonly used in Costa Rica that could also serve as a substrate for the growth of aflatoxigenic fungi include cassava, citrus pulp, banana peel, pineapple shells, and oil palm seeds.

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Crops can be contaminated with AFs in the field, at harvest or during the postharvest stages. In the field, high-temperature stress and drought conditions after *Aspergillus* infection trigger AFs accumulation (Kebede, Abbas, Fisher, & Bellaloui, 2012). During storage, the rate and degree of contamination depend on different factors such as temperature, humidity, water activity, concurrent mycobiota, insect damage, and grain physical injury (EFSA, 2007).

The innocuity of cereal grain-based products for animals and humans should be ensured during processing and throughout the entire food chain using the "farm to fork" models (Yazar & Omurtag, 2008). Aflatoxins are very stable and may resist commonly used food processing techniques like roasting, extrusion, baking, and cooking. For this reason, AFs represent a threat to human and animal health worldwide, and maximum limits (ML) for AFs in food and feed have been established in most countries (García & Heredia, 2014). In 1999, the Costa Rican Ministry of Health set a ML of total AFs of 15 μg kg^{-1} for peanut; and for corn, rice, beans, wheat, oilseeds, legumes, and other cereals a ML of 20 μg kg^{-1}. The ML for AFs for feed and feed ingredients was set at 20 μg kg^{-1} feed.

The concept of One Health recognizes the interconnections between, human, animal and environmental health (Zinsstag, Waltner-Toews, & Tanner, 2015). Under the One Health concept, this interdisciplinary epidemiological study brings together the national surveillance program for animal feed coordinated by the Ministry of Agriculture and Livestock, and the monitoring scheme for agricultural commodities intended for human consumption. Furthermore, information on the incidence of AFs in feed and staple foods in Latin America is scarce, and it is required to estimate the level of exposure of the population to AFs. Therefore, the aim of this study was to determine the occurrence of AFs in agricultural commodities and products intended for animals and for human consumption. Hence, the One Health approach will be applied for the first time to the mycotoxin surveillance in Costa Rica. The results will improve our understanding of the mycotoxin problem in the country and can be used as a tool for decision-making aimed to counteract mycotoxin exposure for both animals and humans.

2. Materials and methods

2.1. Samples

2.1.1. Animal feed and feed ingredient samples

Aflatoxin determinations were conducted in the Microbiology Laboratory of CINA, University of Costa Rica. A total of 970 feedstuffs samples of ca. 5 kg were collected from hay ($n = 322/970$; 33.3%), dairy cow feed ($n = 246/970$; 25.4%), citrus pulp ($n = 40/970$; 4.1%), whole corn ($n = 36/970$; 3.7%), dried distillers grains with solubles (DDGS; $n = 36/970$; 3.7%), calf feed ($n = 36/970$; 3.7%), and different kinds of forages ($n = 31/970$; 3.2%), during 2010–2016 by government inspectors in Costa Rican feed manufacturers, as part of a countrywide surveillance program. Selection of feed and feed ingredients to be tested, number of samples, and sampling sites were chosen by feed control officials, taking into account the most common feedstuff used in Costa Rica, import and export regulations, contamination risk factors, productivity of the feed industry, and the risk for human and animal health associated with each feed or feed ingredient. Sampling was performed following the Association of American Feed Control Officials (AAFCO) recommendations for mycotoxin test object collection (AAFCO, 2017), and samples were taken from silos and storage reservoirs from feed manufacturing plants. All samples were quartered and sieved (1 mm particle size). Fresh material (e.g. forages) was dried at 60 °C before it was processed.

2.1.2. Food commodities for human consumption

A total of 5493 food and agricultural commodities samples intended for human consumption were analyzed during 2003–2015 in the Mycotoxin Laboratory of CIGRAS, University of Costa Rica. The majority of samples corresponded to the most commonly imported commodities for human consumption in Costa Rica (i.e. rice, maize, peanuts, beans, wheat). Sampling was conducted by the State Phytosanitary Service officials in grain shipments at the Pacific Seaport, the Atlantic Seaport, and the Nicaragua border, and sent to CIGRAS for analysis. Other samples analyzed corresponded to products sent by farmers, and the food industry to the Mycotoxin Laboratory for quality control purposes.

2.2. Aflatoxin analysis

2.2.1. Animal feed and feed ingredients

From 2010 to 2011, samples were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA), and from 2012 to 2016 by High Performance Liquid Chromatography (HPLC).

Reagents. An analytical standard with a certified concentration of 2.0 μg mL^{-1}, dissolved in acetonitrile, was purchased from Trilogy® Analytical Laboratory Inc. Linear calibration curves ranging from 0.004 to 0.04 μg mL^{-1} were prepared during quantification. Additionally, a naturally contaminated reference material (TR-MT100, cornmeal, 17.4 μg kg^{-1} of total AFs) was used as a quality control sample (TS-108, Washington, MO, USA). Potassium iodide and metallic iodine (ACS grade), acetonitrile (ACN) and methanol (MeOH, chromatographic grade) were purchased from J.T. Baker (Avantor Materials, PA, USA).

ELISA determinations. A (20.0 ± 0.1) g subsample was used for testing to which 100 mL of an 80:20 MeOH and H_2O solution were added. Measurements were performed according to the ELISA kit manufacturer (AgraQuant® Aflatoxin, Romer Labs®, Getzersdorf, Austria) which has a quantitation range from 1 to 20 μg L^{-1}. Briefly, 100 μL of the methanolic extract, dilution or standard was mixed with 200 μL of conjugate directly in dilution microtitre wells. A 100 μL aliquot of this mixture was added to antibody linked wells and incubated for 15 min. Afterward, 100 μL of the substrate were incorporated, and the mixture was left to stand for 5 min at standard temperature and pressure. Finally, 100 μL of stop solution was added to the mixture. Absorbance measurements were performed immediately using two simultaneous wavelength (450 nm and 620 nm) using a SynergyTM Biotek HT microplate reader and the Gen 5TM software (BioTek Instruments Inc., Winooski, VT, USA).

HPLC determinations. Aflatoxin analysis was performed using a modified ISO/IEC 17025 accredited version of the AOAC method 2003.02. Several modifications were included to span the analysis for other feed and feed ingredients. Briefly, toxin fractions were obtained using an isocrati**c** high-performance liquid chromatography method. Equipment consisted of an Agilent 1260 Infinity series HPLC with a quaternary pump (G1311B), a column compartment (G1316A) kept at 42 °C, a fluorescence detector (G1321B) and an autosampler system (G1329A) set to inject 20 μL (Agilent Technologies, Santa Clara, CA). Peak separation was accomplished using a 5 μm Agilent Zorbax Eclipse C18 column (3.0 mm × 150 mm). The mobile phase was set at a flow rate of 0.8 mL min^{-1} and consisted of H_2O (Type I, TOC 2 μg L^{-1}, 0.055 μS cm^{-1}), MeOH and ACN 60:30:5. Fluorescent derivatives of AFB_1, AFB_2, AFG_1, and AFG_2 were generated with an aqueous 1.2 mmol L^{-1} KI and 0.79 mmol L^{-1} I_2 solution at a flow rate of 0.3 mL min^{-1} at 95 °C using a 0.15 mL reactor on a Pinnacle PCX system (Pickering Laboratories, Mountain View, CA, USA). These derivatives emit light at 435 nm and after excitation at 365 nm.