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Occurrence of mycotoxins in extruded commercial dog food

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ABSTRACT

The aim of this study was to determine the presence and the level of contamination of the most important mycotoxins (deoxynivalenol, fumonisin B_1 and B_2, aflatoxin B_1, B_2, G_1 and G_2, ochratoxin A and zearalenone) in 48 samples of extruded dry dog food found in the Italian market (24 samples from standard economy lines, 24 of premium lines). Analyses were performed using ultra-performance liquid chromatography coupled to tandem mass spectrometry. Although the concentrations of the mycotoxins in all samples proved to respect the European legislation with regards to animal feed, the analyses revealed a substantial presence of deoxynivalenol, fumonisins and ochratoxin A, with values above the limit of quantification (5 μg/kg) in 100%, 88% and 81% of the samples, respectively. In contrast, aflatoxins and zearalenone contamination proved to be very modest, with 88% and 75% of the samples, respectively, showing concentrations below the corresponding limit of quantification (5 μg/kg for aflatoxins and 10 μg/kg for zearalenone). Moreover, despite a very heterogeneous contamination, the concentration of fumonisins and ochratoxin A was significantly higher in standard foods than in premiumones (491 *vs.* 80.2 μg/kg dry matter for fumonisin B_1; 113 *vs.* 38.5 μg/kg dry matter for fumonisin B_2; 599 *vs.* 103 μg/kg dry matter for total fumonisins; 23.8 *vs.* 13.0 μg/kg dry matter for ochratoxin A; $P<0.001$). Furthermore, a simultaneous presence of different mycotoxins (at concentrations higher than their limit of quantification) was observed in most of the pet foods analyzed; in particular, 19% of the samples were contaminated by no fewer than two different types of mycotoxins, 52% by three, 25% by four and 2% by all the mycotoxins evaluated. These results revealed the need for further investigation into the potential risk deriving from chronic exposure to low doses of the different types of mycotoxins that pet species are subject to today.

1. Introduction

Food quality and safety have presently gained considerable importance in the public opinion. In the veterinary field the need to ensure the safety of products of animal origin is reflected nowadays by the routine of performing rigorous tests on

Abbreviations: AFB_1, aflatoxin B_1; AFB_2, aflatoxin B_2; AFG_1, aflatoxin G_1; AFG_2, aflatoxin G_2; BW, body weight; DM, dry matter; DON, deoxynivalenol; FB_1, fumonisin B_1; FB_2, fumonisin B_2; LC-MS, liquid chromatography coupled to mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; MRM, multiple reaction monitoring; OTA, ochratoxin A; RO, reverse osmosis; UP, ultra pure; UPLC-MS/MS, ultra-performance liquid chromatography coupled to tandem mass spectrometry; ZEA, zearalenone.

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feeds intended for livestock animal species, as the foods derived from them represent potential vehicles of substances that are hazardous to humans (EC, 2004).

In consideration of the recent strengthening of the human-pet bond and increased health awareness, as well as the more general concern for pet welfare (Walsh, 2009), the issue of pet food quality and safety is significantly impacting the pet food industry, which today plays a role of considerable importance in so far as the nutritional management of pets is concerned (Assalco, 2014). In this area, mycotoxin contamination, in particular, is drawing increasing interest.

The traditional use of a large quantity of vegetable ingredients and by-products (cereals, for example) by pet food manufacturers, particularly in the formulations of dry products, has enormously favored the risk of mycotoxin intoxication in pet species (Leung et al., 2006; Boermans and Leung, 2007), given that the various steps of the pet food production process are not able to completely inactivate these fungal metabolites (Bullerman and Bianchini, 2007).

In the recent past, some monitoring initiatives carried out in different parts of the world have revealed a significant presence of mycotoxins in the pet food samples analyzed. More specifically, the principal mycotoxins investigated were aflatoxins, fumonisins, deoxynivalenol (DON), zearalenone (ZEA) and ochratoxin A (OTA) (Leung et al., 2006; Boermans and Leung, 2007; Songsermsakul et al., 2007; Böhm et al., 2010; Pagliuca et al., 2011).

With regard to the legislative and regulatory sphere, the situation on an international level is still not sufficiently defined and harmonized. In fact, the reference provisions are mostly aimed at food and feed intended for humans and livestock animals, rather than pet species, with ample variability in terms of tolerance limits among the numerous countries concerned (Mazumder and Sasmal, 2001; EC, 2002, 2006; FAO, 2004; van Egmond et al., 2007).

Although the knowledge about the toxicological effects of mycotoxins in dogs and cats is still limited, some studies have shown that the presence of such substances in pet food can cause serious harm to pet health, with both acute and chronic forms of intoxication depending on the level of contamination and length of exposure (Leung et al., 2006; Boermans and Leung, 2007; Newman et al., 2007; Dereszynski et al., 2008; Bruchim et al., 2012; Wouters et al., 2013).

This study was aimed at identifying and quantifying the main mycotoxins considered under European legislation in complete industrial dry dog foods available in the Italian market and belonging to different price ranges.

2. Materials and methods

2.1. Sampling

Forty-eight complete commercial extruded dry dog foods were purchased from stores in the province of Bologna (Italy). Specifically, the products included 24 low/standard dog foods (consisting in economical formulations ranging in price from €0.80 to 4.00/kg, sold by discount and mass-market retailers) and 24 premium/super premium dog foods (consisting in more costly formulations ranging in prices from €4.00 to 15.00/kg, found in specialized stores). The size of the packages purchased was in the range of 300 g to 5 kg.

Particularly, this reference species was chosen for the study, as dog foods generally contain larger quantities of cereal ingredients than those formulated for the feline species, and thus dogs are likely to be exposed to a greater risk of contamination than cats.

All the analyses were conducted on a representative sample of each product (about half of the content of every package was ground and used for chemical analyses and mycotoxins determination).

2.2. Chemical analyses of the samples

The pet food samples were subjected to chemical analysis to determine moisture and starch content according to the official methods of the Association of Official Analytical Chemists (AOAC, 2000; method 950.46 for moisture and method 996.11 for starch).

2.3. Determination of mycotoxin concentration

2.3.1. Chemicals and reagents

Aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), fumonisinB_1 (FB_1), fumonisin B_2 (FB_2), DON, ZEA and OTA standards were purchased from Sigma–Aldrich (Steinheim, Germany).

U-[$^{13}C_{17}$]-AFB_1, U-[$^{13}C_{34}$]-FB_1, U-[$^{13}C_{15}$]-DON, U-[$^{13}C_{18}$]-ZEA and U-[$^{13}C_{20}$]-OTA were obtained from Romer Lab, Inc.-Biopure (Tulln, Austria).

Methanol and formic acid, used as the mobile phases, and ammonium acetate were of analytical grade specific for liquid chromatography coupled to mass spectrometry (LC-MS) analysis and were purchased from Riedel-de Haën (Seelze, Germany). Acetonitrile and acetic acid, used in the extraction procedures, were purchased from Merck (Darmstadt, Germany).

Reverse osmosis (RO) and ultra pure (UP) water, respectively used as an extraction solvent and mobile phase, were produced by a Human Power apparatus from Human Corporation (Seul, Korea).