Comparison of mycotoxin concentrations in grain versus grain-free dry and wet commercial dog foods

John H. Tegzes\textsuperscript{a}, Brian B. Oakley\textsuperscript{a} and Greg Brennan\textsuperscript{b}

\textsuperscript{a}College of Veterinary Medicine, Western University of Health Sciences, Pomona, California, USA; \textsuperscript{b}Department of Medical Microbiology and Immunology, University of California, Davis, Davis, California, USA

ABSTRACT

Mycotoxins are secondary fungal metabolites that cause both acute and chronic disease in humans and animals. Grains are a common substrate for molds and the production of mycotoxins. This study compared mycotoxin concentrations between grain and grain-free commercial dog foods. In total, 60 samples of dry and wet dog foods produced by five major manufacturers within the US were purchased from pet food retailers in southern California. A standard mycotoxin panel was performed by a reference laboratory in Seattle using industry standard methodology for pet foods. Results of the study demonstrated measurable mycotoxin concentrations in dry dog foods containing grains but not in grain-free dry dog foods, or in wet foods either containing grains or grain-free. This study suggests that the risk of mycotoxin exposure is higher in dry dog foods containing grains. To mitigate this risk, dog food manufacturers could incorporate grains that are categorized as US No. 1 by the USDA and therefore less susceptible to mycotoxin formation.

KEYWORDS

Mycotoxin; dog food; grain; grain-free; fumonisin

Introduction

Grains, particularly corn and wheat, have been used as sources of carbohydrates in pet food formulations for decades [1]. Recently, grain co-products from corn, such as corn gluten meal, or bran derived from whole grains like barley and oats, are also commonly added to pet foods as indigestible fiber sources [1]. In recent years grain-free diets for companion animals have been gaining popularity among pet owners [2, 3] who often choose grain-free diets because of perceived health benefits. Sales of grain-free pet foods increased by 28% in US pet stores during a one-year period from September 2012 to September 2013. In 2015, 45% of all new pet food items introduced were grain-free [2, 3]. Despite this increasing popularity of grain-free diets, there are very few scientific studies determining what, if any, benefits these diets may provide.

One of these perceived health benefits of grain-free diets is the possibility to reduce grain consumption by companion animals, theoretically reducing the risk of potential exposure to mycotoxins [4]. Mycotoxins are secondary metabolites produced by filamentous fungi that can contaminate grains, often due to improper grain storage. The most common contaminants of feed include aflatoxins, fumonisins, ochratoxin A, zearalenone, and the trichothecenes deoxynivalenol, T-2 toxin, and HT-2 toxin. These mycotoxins have a variety of harmful cytotoxic mechanisms [5].

Raw grains, feed ingredients, and finished feed are governed by specific regulatory guidelines [6, 7] but mycotoxin contamination is particularly difficult to avoid because mycotoxins are relatively robust to heat and chemical inactivation processes in downstream processing steps [7, 8]. A study conducted in Poland found multiple mycotoxins in dry veterinary diets for dogs [9]. Among the various mycotoxins detected in the veterinary diets zearalenone was detected in 69% of samples, deoxynivalenol (DON) in 52%, fumonisin B1 in 33%, and nivalenol in 26% [9]. The clinical effects of mycotoxins vary based on type, concentration, and frequency of exposure. Some mycotoxins cause morbidity and mortality both acutely due to high dose exposures and chronically after prolonged low-dose exposures. Effects can include acute toxicosis such as acute hepatic injury presenting as anorexia, depression, gastrointestinal hemorrhage, jaundice or seizures [10, 11]. Chronic diseases such as liver and kidney fibrosis, infections resulting from immunosuppression,
and cancer have been associated with low-dose, chronic mycotoxin exposure [10, 11]. In one clinical study, a combination of mycotoxins including aflatoxin B1, aflatoxin B2, fumonisin B1, fumonisin B2, ochratoxin A, and zearalenone induced immunotoxicity on canine peripheral blood mononuclear cells [5]. Therefore, the potential for mycotoxin contamination in pet food poses a serious health threat [12].

Regulations regarding permissible concentrations of mycotoxins in animal feeds focus mainly on farm animals used for food production. While much of what is known about mycotoxins in animals is based on toxicological data demonstrating adverse effects in farm and laboratory animals exposed to naturally occurring concentrations of mycotoxins, there is perhaps even more concern for companion animals who are often maintained and fed for longer periods of time on a homogeneous, grain-containing diet and thus more likely to have chronic exposures to pet foods contaminated with either single mycotoxins, or multiple mycotoxins in various combinations [4]. Maximum concentrations permitted in pet foods are generally extrapolated from a generalized “other animal” category, meaning non-food animal guidelines rather than pet-specific regulations [4]. However, these concentrations do not necessarily indicate “safe levels” for mycotoxin exposure in companion animals [13] since very few studies have been conducted in pets. Moreover, none of these studies have investigated the long-term chronic exposures that likely occur if pets are fed a contaminated feed over a typical lifespan [4, 13]. Due to this uncertainty, one of the perceived health benefits of grain-free diets might be due to the elimination of low-dose chronic exposures to mycotoxins, as grains in pet food are presumed to be the main source of mycotoxin contamination [4]. However, the true prevalence of mycotoxins in either grain-containing or grain-free pet food has not been systematically examined.

While there have been only a few reports, previous studies have detected mycotoxins in dog foods, especially dry foods. Dry dog foods contain 3–11% moisture, while wet dog foods contain 60–87% moisture [14]. DON contamination was common in studies conducted in Austria [15] and Italy [16]. In both of these studies, measurable concentrations of DON were detected in all dry food samples. A broad range of DON concentrations in dry food were found in the Austrian study and 27% of wet food samples also contained detectable concentrations of DON [15]. A second study conducted in Austria found similar patterns of DON, fumonisins, and zearalenone [4].

A study conducted in Brazil also reported low-levels of multiple mycotoxins in dry dog foods [8]. In all four of these studies mycotoxins produced by Fusarium species of fungi were most common, often contaminating the same dog food with three different types of mycotoxins [8].

In the current study, we sampled wet and dry commercial dog foods produced by five different manufacturers with and without grains and measured the concentrations of 11 mycotoxins in these samples.

**Methods**

Samples were obtained from five different brands in an attempt to broadly sample commercially available dog food from different supply chains. All pet foods sampled were manufactured in the U.S. following US guidelines for the manufacturing of dog food. Formulations from the same brand were paired such that the only significant difference in the ingredients list was the presence or absence of grain. For example, adult maintenance formulations were chosen by the same product name clearly labeled as either containing grains, or as grain-free, ensuring brand consistency across all paired samples. Six dry food (three containing grains and three grain-free) and six wet food samples (three containing grains and three grain-free) were obtained for each manufacturer except for one manufacturer that does not formulate any dry foods. Because the number of samples was constrained by cost, we ignored brand designation in all analyses, clustering the samples only as dry versus wet food and grain-containing or grain-free food.

A total of 60 dog food samples were analyzed for 11 different mycotoxins using stable isotope dilution LC-MS/MS methodology at IEH Laboratories (Seattle, WA) [17, 18]. Analytical detection limits ranged from 1.0 ppb to 0.10 ppm depending on the mycotoxin (Table 1).

| Mycotoxin          | Reporting Limit |
|--------------------|-----------------|
| Aflatoxin B1      | 1.0 ppb         |
| Aflatoxin B2      | 1.0 ppb         |
| Aflatoxin G1      | 1.0 ppb         |
| Aflatoxin G2      | 1.0 ppb         |
| Deoxynivalenol    | 0.10 ppm        |
| Fumonisin B1      | 0.10 ppm        |
| Fumonisin B2      | 0.10 ppm        |
| HT-2 Toxin        | 0.10 ppm        |
| Ochratoxin A      | 2.0 ppb         |
| T-2 Toxin         | 0.10 ppm        |
| Zearalenone       | 20 ppb          |
Results

Only dry dog foods containing grains had detectable mycotoxin contamination, and only mycotoxins that are products of the *Fusarium* genus were detected (Table 2). Of the 12 dry dog foods containing grains that were analyzed, nine of the twelve had at least one detectable *Fusarium* mycotoxin (Table 3). For DON and fumonisin B1, 9/12 dry grain foods were above detection limits while 8/12 samples were positive for fumonisin B2 and 4/12 samples tested positive for zearalenone (Table 3). For DON and fumonsin B1, 9/12 dry grain foods were above detection limits while 8/12 samples were positive for fumonsin B2 and 4/12 samples tested positive for zearalenone (Table 3). These results are consistent with findings from studies conducted in Austria [4, 15], Italy [16], and Brazil [8]. None of the 60 samples tested had concentrations above the detection limits for aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, HT-2 toxin, ochratoxin A, or T-2 toxin. When considered by brand, at least one of the four *Fusarium* mycotoxins was found in each of the four brands of dry grain foods (Table 4). For two brands (Brand 4 and Brand 5), at least one of the three samples tested were positive for all four *Fusarium* mycotoxins (Table 4).

The US Food and Drug Administration (FDA) has published guidance concentrations for pet foods, but not regulatory limits, for aflatoxin, fumonisin, and DON [6]. The guidance concentration is 10 ppm for total fumonisins (fumonisin B1 + fumonisin B2 + fumonisin B3). The concentrations detected in this study were below the 10 ppm guidance value (Figures 1–4).

Discussion

In this study, we identify low-level *Fusarium*-derived mycotoxin contamination in grain-containing dry dog food but did not detect any mycotoxin contamination in either grain-free dry dog food or wet dog food. In addition, none of the analyzed samples contained aflatoxins in detectable concentrations, which may reflect how regulatory and control strategies have been effective in reducing the incidence of aflatoxins in dry commercial dog foods. The presence of *Fusarium* mycotoxins highlights the need to establish similar control strategies targeting these mycotoxins, especially for the manufacture of dry dog foods. We found *Fusarium*-derived mycotoxin concentrations well below amounts considered to be acutely toxic to dogs, but these data support the possibility that feeding grain-containing pet food may result in chronic exposure to a variety of mycotoxins. The effects of chronic low-level mycotoxin exposure in dogs remain unknown but merit further study [4].

Dry dog foods contain higher amounts of grains than wet dog foods, potentially explaining the higher levels of mycotoxins in grain-containing foods. Grains in these dog foods are the most likely source of mycotoxin contamination, although we cannot be certain of the source because we only analyzed end products in this study. When grains are incorporated into dog food formulations it is important that high quality grain is used. Grain quality is correlated with

### Table 2. Number of samples with values above the detection threshold for deoxynivalenol, fumonisin B1, fumonisin B2, and zearalenone respectively, according to grain and food type for all samples.

|           | Dry | Wet |
|-----------|-----|-----|
| Grain     | 9, 9, 8, 4 (n = 12) | 0, 0, 0, 0 (n = 18) |
| Grain-free| 0, 0, 0, 0 (n = 12) | 0, 0, 0, 0 (n = 18) |

For the only category with positive values (Dry Grain; shown as shaded) the data are shown in more detail by brand in Table 4.

### Table 3. Concentration ranges and number of positive samples for mycotoxins detected in dry dog food.

| Dry Dog Food | Deoxynivalenol (0.10 ppm) | Fumonisin B1 (0.10 ppm) | Fumonisin B2 (0.10 ppm) | Zearalenone (20 ppb) |
|--------------|---------------------------|-------------------------|-------------------------|---------------------|
| Grain (n = 12) | n = 9                     | n = 9                   | n = 8                   | n = 4               |
| Concentration Range | 0.12–0.32 ppm           | 0.32–3.2 ppm            | 0.21–1.6 ppm            | 24–65 ppb           |
| Grain-free (n = 12) | 0                        | 0                      | 0                      | 0                   |

Analytical detection limits are shown in parentheses for each mycotoxin.

### Table 4. Number of samples with values above the detection threshold for deoxynivalenol, fumonisin B1, fumonisin B2, and zearalenone respectively, according to brand for dry grain samples.

| Brand | Dry, Grain |
|-------|------------|
|       |            |
| Brand 1 | 3, 0, 0, 0 (n = 3) |
| Brand 2 | n = 0      |
| Brand 3 | 3, 3, 2, 0 (n = 3) |
| Brand 4 | 2, 3, 3, 3 (n = 3) |
| Brand 5 | 1, 3, 3, 1 (n = 3) |

For Brand 2, no dry grain product is sold. For each of the four other brands three samples were tested. N/A = not applicable.

**Figure 1.** Deoxynivalenol [19]
mycotoxin contamination as lower grade grains often contain broken and fragmented grains which are much more susceptible to mold growth and subsequent mycotoxin production [23]. Grains are numerically graded based on factors such as test weight, proportion of damaged or broken kernels, presence of foreign odors, or heat-damage [24]. Any of these factors can contribute to mold growth and mycotoxin production. However, pet food manufacturers may choose grains unfit for human consumption as a cost-cutting strategy [25]. Using only grains graded as US No. 1 by the USDA could be a control strategy to minimize mycotoxin contamination from ingredients incorporated into pet food. Currently, there is no requirement to reveal the grade of grain incorporated into pet food, but noting the grade of grains used on the ingredients list could help consumers choose pet foods with more confidence.

We did not test any cat foods in this study. Cats are obligatory carnivores and grains are less frequently incorporated into dietary formulations. It has been proposed that finding measurable mycotoxins in cat foods is indicative of high grain content [25]. Further studies could assess the frequency of mycotoxins found in cat foods and correlate findings with the presence of grains.

While the results of this study might suggest that grain incorporation into dog food formulations has risks, we do not conclude that it is inappropriate to use them in dog foods. Grain-free dog foods need to be carefully formulated to meet nutritional requirements. A recent study demonstrated evidence of partially reversible cardiomyopathy in some dogs fed grain-free diets. While the exact associations with grain-free diets remains unclear, the data suggested that the condition could be reversed after a diet change [26]. Additionally, more work needs to be done to assess the effects of mycotoxins in combinations and in low, chronic concentrations in dogs, perhaps studying them in multi-year long-term research studies. Also, there is a need to continue to explore strategies to minimize the concentrations of all mycotoxins in the manufacturing of dog food. Perhaps requiring grain grades on ingredient lists could increase understanding about the potential sources of mycotoxins, inform consumer choice, and provide insights to other strategies to further minimize contamination.
Disclosure statement
JT has a financial interest as a shareholder with Just Food For Dogs.

Funding
Just Food For Dogs & Western University of Health Sciences: This work was partially funded by Just Food For Dogs, Irvine, CA, USA under Grant 25239 and Western University of Health Sciences.

Notes on contributors

John Tegzes, MA, VMD, Diplomate ABVT, is a veterinarian with specialty board certification in Toxicology by the American Board of Veterinary Toxicology. He is a Professor of Toxicology in the College of Veterinary Medicine at Western University of Health Sciences, where he also serves as the Director of Interprofessional Practice and Education for the university. He has previously served as a Specialist in Poison Information at the Oregon Poison Center and the California Poison Control System.

Brian Oakley, PhD, is Associate Professor of Microbial Ecology in the College of Veterinary Medicine at Western University of Health Sciences. He currently serves as Interim Director of Year 3 of the DVM curriculum. Prior to joining the WesternU faculty in 2014, he was a Research Microbiologist with the USDA Agricultural Research Service in Athens, GA.

Greg Brennan, DVM, PhD, is a Veterinarian and Immunologist and currently serves as Project Scientist in the Rothenburg Lab in the School of Medicine, Department of Medical Microbiology and Immunology at the University of California, Davis. Previously he was Assistant Professor in the College of Veterinary Medicine at Western University of Health Sciences.

ORCID

John H. Tegzes http://orcid.org/0000-0002-8771-4705
Greg Brennan http://orcid.org/0000-0002-4339-9045

References
[1] De Godoy MRC, Kerr KR, Fahey GC. Alternative dietary fiber sources in companion animal nutrition. Nutrients. 2013;5:3099–3117.
[2] Deng P, Swanson KS. COMPANION ANIMALS SYMPOSIUM: Future aspects and perceptions of companion animal nutrition and sustainability. J Anim Sci. 2015;93:823–834.
[3] Petfood Industry.com: Data from GfK show 28 percent spike in grain-free petfood sales [Internet]. 2013. November 13 [cited 2018 Dec 14] Available from: https://www.petfoodindustry.com/articles/3992-data-from-gfk-show-28-percent-spike-in-grain-free-petfood-sales.
[4] Boehm J, Koinig L, Razzazi-Fazeli E, et al. Survey and risk assessment of the mycotoxins deoxynivalenol, zearalenone, fumonisins, ochratoxin A, and aflatoxins in commercial dry dog food. Mycotox Res. 2010;26:147–153.
[5] Singh SD, Sheik Abdul N, Phulukdaree A, et al. Toxicity assessment of mycotoxins extracted from contaminated commercial dog pelleted feed on canine blood mononuclear cells. Food Chem Toxicol. 2018;114:112–118.
[6] United States Food & Drug Administration (FDA): Guidance for industry: Fumonisins levels in human foods and animal feeds [Internet]. Washington (DC): November 2001. [cited 2019 Jun 19]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-fumonisin-levels-human-foods-and-animal-feeds.
[7] Marin S, Ramos AJ, Cano-Sancho G, et al. Mycotoxins: Occurrence, toxicology, and exposure assessment. Food Chem Toxicol. 2013;60:218–237.
[8] Bissoqui LY, Frehse MS, Freire RL, et al. Exposure assessment of dogs to mycotoxins through consumption of dry feed. J Sci Food Agric. 2016;96:4135–4142.
[9] Witaszak N, Stepien Ł, Bocianski J, et al. Fusarium species and mycotoxins contaminating veterinary diets for dogs and cats. Microorganisms. 2019;7:26.
[10] Meerdink GL. Aflatoxins. In: Plumlee KH, editor. Clinical veterinary toxicology. St. Louis: Mosby; 2004. p. 231–235.
[11] Coppock RW, Christian RG. Aflatoxins. In: Gupta RC, editor. Veterinary toxicology. New York: Elsevier; 2007. p. 939–950.
[12] Boermans HJ, Leung MC. Mycotoxins and the pet food industry: Toxicological evidence and risk assessment. Int J Food Microbiol. 2007;119:95–102.
[13] Leung MC, Díaz-Llano G, Smith TK. Mycotoxins in pet food: a review on worldwide prevalence and preventative strategies. J Agric Food Chem. 2006;54:9623–9635.
[14] Crane SW, Cowell CS, Stout NP, et al. Commercial pet foods. In: Hand MS, Thatcher CD, Remillard RL, Roudebush P, Novotny BJ, editors. Small animal clinical nutrition, 5th Edition. Topeka: Mark Morris Institute; 2010. p. 157–190.
[15] Songsermsakul P, Razzazi-Fazeli E, Boehm J, et al. Occurrence of deoxynivalenol (DON) and ochratoxin A (OTA) in dog foods. Mycotoxin Res. 2007;23:65–67.
[16] Gazzotti T, Biagi G, Pagliuca G, et al. Multi-mycotoxin stable isotope dilution assay (MS) for Fusarium toxicins cereals. Anal Bioanal Chem. 2016;408:235–248.
[17] Habler M, Rychlik M. Multi-mycotoxin stable isotope dilution LC-MS/MS method for Fusarium toxicins cereals. Anal Bioanal Chem. 2016;408:307–317.
[18] Rychlik M, Asam S. Stable isotope dilution assays in mycotoxin analysis. Anal Bioanal Chem. 2008;390:153–167.
[19] ChemSpider: Deoxynivalenol. London, UK: Royal Society of Chemistry; 2015. Available from: http://www.chemspider.com/Chemical-Structure.36584.html?rid=cab43d7d-508c-4db3-a4c3-63d9a418030a.
[20] ChemSpider: Fumonisin B1. London, UK: Royal Society of Chemistry: 2015. [cited 2019 Jun 19]. Available from: http://www.chemspider.com/Chemical-Structure.2015282.html?rid=a49b9aed-ed0d-45ac-a291-d2194de15699&page_num=0.

[21] ChemSpider: Fumonisin B2. London, UK: Royal Society of Chemistry: 2015. [cited 2019 Jun 19]. Available from: http://www.chemspider.com/Chemical-Structure.2015284.html?rid=7386bd22-e3c6-428f-808d-8f14979cd74b.

[22] ChemSpider: Zearalenone. London, UK: Royal Society of Chemistry: 2015. [cited 2019 Jun 19]. Available from: http://www.chemspider.com/Chemical-Structure.4444897.html?rid=59c201d7-96a5-4c84-b872-dd4f5c5ff8a&page_num=0

[23] United States Department of Agriculture (USDA): Mycotoxin handbook. Washington (DC); 2015. [cited 2018 Dec 21]. Available from: https://www.gipsa.usda.gov/fgis/handbook/MycotoxinHB/MycotoxinHandbook_2016-07-12.pdf.

[24] United States Department of Agriculture (USDA): Official United States Standards for Grain. Washington (DC): Federal Grain Inspection Service; 2007. [cited 2018 Dec 20]. Available from: https://www.gipsa.usda.gov/fgis/usstandards.aspx.

[25] Singh SD, Baijnath S, Chuturgoon AA. A comparison of mycotoxin contamination of premium and grocery brands of pelleted cat food in South Africa. J S Afr Vet Assoc. 2017;88:e1–e4. https://doi.org/10.4102/jsava.v88i0.1480.

[26] Adin D, DeFrancesco TC, Keene B, et al. Echocardiographic phenotype of canine dilated cardiomyopathy differs based on diet type. J Vet Cardiol. 2019;21:1–9.