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REVIEWS

Mycotoxins in Pet Food: A Review on Worldwide Prevalence and Preventative Strategies

MAXWELL C. K. LEUNG, GABRIEL DÍAZ-LLANO, AND TREVOR K. SMITH*

Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Mycotoxins contaminate cereal grains worldwide, and their presence in pet food has been a potential health threat to companion animals. Aflatoxins, ochratoxin A, and *Fusarium* mycotoxins have been found in both raw ingredients and final products of pet food around the globe. Aflatoxin, a hepatotoxin and carcinogen, has caused several food poisoning outbreaks in dogs, and aflatoxin content is regulated in pet food in many countries. Ochratoxin A and *Fusarium* mycotoxins including trichothecenes, zearalenone, and fumonisins may have chronic effects on the health of companion animals. Grain processing, sampling error, analytical methods, conjugated mycotoxins, storage conditions, and synergistic interactions are common challenges faced by the pet food industry. Food-processing techniques such as sieving, washing, pearling, ozonation, and acid-based mold inhibition reduce the mycotoxin content of cereal grains. Dietary supplementation with large neutral amino acids, antioxidants, and omega-3 polysaturated fatty acids as well as inclusion of mycotoxin-sequestering agents and detoxifying microbes may ameliorate the harmful effects of mycotoxins in contaminated pet food.

Keywords: Mycotoxins; pet food; surveys; feed sampling; analytical techniques; food processing; nutrient supplementation; mycotoxin-sequestering agent; microbial detoxification

1. INTRODUCTION

Mycotoxins are secondary fungal metabolites (i.e., metabolites not essential to the normal growth, development, and reproduction of fungus) that cause pathological changes in animal species (*1*). They are low molecular weight compounds with diverse chemical structures and biological properties (**Figure 1**). A large number of fungal metabolites have been identified as mycotoxins, contaminating agricultural commodities worldwide. *Fusarium*, *Pencillium*, and *Aspergillus* are the three most important mycotoxin-producing fungal genera with respect to animal and human health (*2*). Fungal invasion and toxin production in crops can occur preharvest, at harvest, and during processing, transportation, and storage. Temperature and moisture can greatly affect the growth rate of fungi as well as the types and amounts of mycotoxins produced (*1*).

Mycotoxins have attracted worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity, and both domestic and international trade (*3*). It has been estimated that the impact of mycotoxins on the feed and livestock industries causes annual losses of $5 billion in the United States and Canada (*3*).

Because cereal grains and nuts are often used as ingredients in commercial pet food, companion animals such as cats, dogs, birds, rabbits, and guinea pigs are often exposed to the effects of mycotoxins. Cereal byproducts, furthermore, may be diverted to animal feed even though they may contain concentrated levels of mycotoxins compared to raw cereals (*4*, *5*).

There have been several reviews of the physiological effects of mycotoxins in pet species in recent years (*6*−*8*). The current review, in contrast, addresses the worldwide prevalence of mycotoxin contamination in pet food and technological advances in preventing mycotoxicoses in companion animals. Mycotoxin outbreaks in companion animals, mycotoxin surveys of commercial pet food, challenges faced by the pet food industry, and some practical preventive strategies for mycotoxicoses will be discussed.

2. MYCOTOXINS IN PET FOOD

Mycotoxins commonly found in pet food include aflatoxins, ochratoxins, and the *Fusarium* mycotoxins. They differ in their toxic effects and their prevalence across regions, entering feed-manufacturing processes through contaminated raw materials (**Table 1**). These mycotoxins may have chronic effects on animal health. When highly contaminated ingredients are accidentally used in feed production, animals can develop acute mycotoxicosis (**Table 2**). Aflatoxins, ochratoxins, and tremor-

* Corresponding author [telephone (519) 824-4120, ext. 53746; fax (519) 822-7897; e-mail tsmith@uoguelph.ca].

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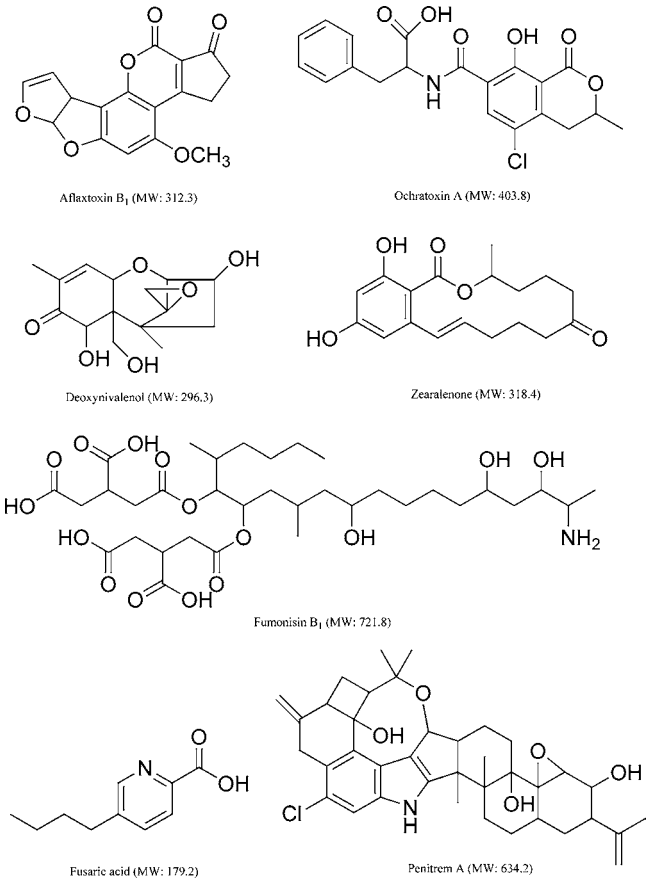

Figure 1. Structures of major mycotoxins.

genic myocotoxins have also resulted in several food-poisoning cases in pet animals due to the consumption of spoiled foods.

2.1. Aflatoxins. Aflatoxins are a group of mycotoxins produced by *Aspergillus* spp., mainly *A. parasiticus* and *A. flavus* (*2*). They are common fungal contaminants of nuts but are also found in many other feedstuffs (**Table 1**). Aflatoxins B_1, B_2, G_1, and G_2 are four naturally occurring forms of aflatoxins, with aflatoxin B_1 being the most potent, prevalent, and carcinogenic (*6*, *9*).

The chemical structure of aflatoxin incorporates dihydrofuran and tetrahydrofuran moieties coupled to a substituted coumarin (*10*) (**Figure 1**). Aflatoxins may be converted to more reactive, electrophilic epoxides by phase I metabolism occurring primarily in the liver (*1*). The unstable epoxides may bind covalently to intracellular macromolecules such as DNA, RNA, and protein, resulting in adduct formation, cellular damage, and hepatic necrosis (*11*).

Aflatoxins are hepatotoxic and carcinogenic. Dogs exposed to 0.5−1 mg of aflatoxin/kg of body weight (bw) typically die within days, exhibiting vomiting, depression, polydipsia, polyuria, and hepatitis (*7*). Anorexia, lethargy, jaundice, disseminated intravascular coagulation, and death have also been described in dogs exposed to 0.05−0.3 mg of aflatoxin/kg of feed over 6−8 weeks. Newberne and Wogan (*12*) experimentally induced malignant tumors in rats with an 80-week exposure to 15 μg of aflatoxin B_1/kg of feed. The sensitivity of companion animals to aflatoxin B_1, expressed as oral LD_{50}, varies considerably, with rabbits (0.3 mg/kg of bw), mink (0.5−0.6 mg/kg of bw), cats (0.55 mg/kg of bw), dogs (1.0 mg/kg of bw), and guinea pigs (1.4−2.0 mg/kg of bw) being more sensitive than mice (9.0 mg/kg of bw) or hamsters (10.2 mg/kg of bw) (*10*).

Many food-poisoning outbreaks in dogs have been associated with aflatoxin contamination because aflatoxins have a high toxicity in canine species (**Table 2**). Anorexia and depression are the two most visible symptoms of aflatoxicosis in dogs, although sudden deaths are also often reported. Dogs are typically exposed to aflatoxins through accidental inclusion of contaminated corn in the feed-manufacturing processes (*13*−*19*) and, in some cases, through improper preparation of domestically prepared dog food (*20*−*22*). In the former cases, aflatoxin outbreaks may persist for up to several months before being diagnosed, thereby affecting a large number of animals (*16*, *17*, *19*). Early diagnosis by veterinarians and prompt recall procedures are crucial in controlling such outbreaks.

Aflatoxins have been detected in commercial pet food worldwide, notably in North and South America (**Table 3**). Wild bird feed was found to be the most contaminated among different types of pet foods in several surveys, possibly due to the use of corn, nuts, and seeds as significant ingredients (*23*−*25*). Up to one-fourth of the wild bird feed samples were contaminated with more than 100 μg of aflatoxin B_1/kg in two surveys. This presents a potential health threat to the birds (*24*, *25*).

Commercial dog and cat foods, in contrast, generally have lower aflatoxin content. Although the percentage of aflatoxin-positive samples varies by survey, almost all of the positive samples contained <20 μg of aflatoxin B_1/kg (*23*, *24*, *26*−*28*). Such a low level of aflatoxin exposure appears to be insufficient to cause noticeable symptoms in companion animals, but the chronic hepatotoxic and carcinogenetic effects should not be overlooked.

A number of aflatoxin-producing fungi have also been detected in commercial pet food (*29*, *30*). *Aspergillus flavus*, for example, was found in 14 of 60 pet food samples purchased in Portugal (*30*). Scudamore et al. (*23*), in another study, identified significant growth of *Aspergillus* spp. in commercial pet food with 20−25% moisture content after a 4-week incubation. Although the presence of toxigenic fungi does not necessarily result in mycotoxin production, preventing fungal growth in pet food can certainly minimize the risk of mycotoxicoses. Bueno et al. (*29*) proposed that a moisture content below 11.50% could suppress fungal growth in pet food.

2.2. Ochratoxins. Ochratoxins are produced by numerous *Aspergillus* and *Penicillium* spp., including *A. ochraceus* and *P. verrucosum* (*10*). Ochratoxins are found principally in cereal grains but also in animal byproducts because of their tight binding with plasma proteins and long half-life in animal tissues (**Table 1**). There are four ochratoxin homologues: A, B, C, and D. Ochratoxin A is the most prevalent, whereas ochratoxins A and C are the most toxic (*1*).

Ochratoxins are phenylalanine-containing dihydroisocoumarins (**Figure 1**). Although their mechanism of action remains unclear, it has been suggested that ochratoxins act by disrupting phenylalanine metabolism (*31*). Ochratoxin A also possesses a chlorinated phenol moiety, a procarcinogenic group that causes electrophilic attack on DNA (*28*). Ochratoxins accumulate primarily in the kidneys as a result of high blood flow and tubular re-uptake (*32*). Ochratoxin A has also been found to interfere with signal transduction pathways at nanomolecular concentration in renal cells, leading to specific changes in function and phenotype but not to necrosis (*33*).

Ochratoxin A is a potent nephrotoxin in pet species. A 2-week exposure to 0.3 mg of ochratoxin A/kg of bw is lethal to young male beagle dogs, which exhibit severe kidney damage, anorexia, vomiting, weight loss, tenesmus, intestinal hemorrhage, tonsillitis, dehydration, and prostration (*34*). A 1-year exposure to 0.8 mg of ochratoxin A/kg of feed caused mild nephropathy

Table 1. Regional Prevalence and Sources in Feedstuffs of Major Mycotoxins

mycotoxin	regions of high prevalence	sources in feedstuffs	refs
aflatoxins	temperate and tropical regions: North and South America, southern Asia, and Africa	corn, peanuts, cottonseed, tree nuts, wheat, and rice	*1*, *10*, *164*
ochratoxins	temperate regions: Balkans, northern Europe, and Canada	barley, corn, wheat, oats, nuts, dried beans, and animal byproducts	*1*, *10*
Fusarium mycotoxins	temperate regions: Europe, United States, Canada, Russia, Japan, China, Brazil, Argentina	corn, barley, wheat, oats, and soybean	*1*, *3*, *175*

in pigs (*35*). In addition to acute and chronic nephrotoxic effects, ochratoxin A has also been shown to be immunotoxic, teratogenic, and nephrocarcinogenic in animals (*8*, *32*, *36*, *37*).

Although ochratoxin A is widely found in pet food (**Table 3**) and has a long half-life in vivo, the significance of the effect of ochratoxin A on the health of pet animals remains unclear. Several studies in Europe revealed that most pet food did not have a detectable amount of ochratoxin A or contained <5 μg of ochratoxin A/kg (*23*, *30*, *38*, *39*), and the chronic effect of such a low dietary exposure has not been investigated in companion animals. Pühringer et al. (*39*) investigated the ochratoxin A content of 101 feline kidneys in Austria, and 16 of the kidney samples contained 0.31−5.18 μg of ochratoxin A/kg. This finding suggested a high dietary ochratoxin exposure in the cats but did not correlate with the pathological findings in the kidney samples. Occasional cases of acute ochratoxicosis in companion species have been reported in the literature (*40*−*42*; **Table 2**).

2.3. *Fusarium* Mycotoxins. The *Fusarium* mycotoxins are a chemically and biologically diverse group of compounds (**Figure 1**). The trichothecenes, zearalenone, and fumonisins are considered to be particularly important in animal health worldwide (*43*). The trichothecenes, a family of over 100 tetracyclic sesquiterpenoid compounds including deoxynivalenol, diacetoxyscirpenol, and T-2 toxin, can be produced by *F. graminearum*, *F. sporotrichioides*, and *Stachybotrys chartarum* (*44*). Zearalenone is produced primarily by *F. graminearum*, *F. avenaceum*, and *F. nivale*. Fumonisin and fusaric acid are produced by a variety of *Fusarium* species.

Many cereal ingredients of pet food are known to contain the *Fusarium* mycotoxins. Corn, for example, is commonly contaminated with fumonisins (*45*−*49*). Corn, wheat, and barley produced in North America are often contaminated with deoxynivalenol, the most common trichothecene (*50*, *51*).

The *Fusarium* mycotoxins have different modes of action. Trichothecenes can cause vomiting, feed refusal, gastrointestinal irritation, and immunosuppression (*1*). Hughes et al. (*52*) reported anorexia and vomiting in dogs and cats exposed to 4.5 and 7.7 mg of deoxynivalenol/kg of feed, respectively. Zearalenone, on the other hand, is an estrogenic mycotoxin (*53*). A 7-day dietary exposure to 200 μg of zearalenone/kg of bw/day was found to produce pathological changes in the canine reproductive system (*54*, *55*). Fumonisins inhibit sphingolipid synthesis and metabolism and damage various organs in animals (*1*). Equine leukoencephalomalacia can be caused by <5 mg of fumonisin B_1/kg of feed and is characterized by feed refusal, depression, ataxia, blindness, and hysteria (*1*). Fumonisin B_1 was also found to cause liver and kidney cancer in rodents (*56*). Fusaric acid is a potent inhibitor of dopamine-β-hydroxylase, suppressing norepinephrine synthesis in the brain of dogs, cats, rabbits, and rats (*57*−*59*). Reduced appetite, vomiting, suppressed weight gain, and hypotension were observed after the feeding of young beagle dogs 50 mg of fusaric acid/kg of bw for 30 days and 6 months (*60*, *61*).

Despite their marked prevalence in the cereal ingredients of pet foods, less research has been devoted to the *Fusarium* mycotoxins compared to aflatoxins and ochratoxins. A number of surveys, however, have shown that they are present in significant amounts in commercial pet food (**Table 3**). Zearalenone and fumonisin B_1, for instance, were found in 84 and 100% of samples, with the highest levels being 299.5 and 1410 μg/kg of feed respectively (*62*, *63*). Although acute *Fusarium* mycotoxicoses have not been reported to date in pet species, the chronic toxicity to pet animals should be of concern.

2.4. Tremorgenic Mycotoxins. Tremorgenic mycotoxins are produced by *Penicillium*, *Aspergillus*, and *Claviceps* (*6*). Unlike aflatoxins, ochratoxin A, and the trichothecenes, tremorgenic mycotoxins are rarely found in feed ingredients but are usually produced during food spoilage. Dogs are exposed to tremorgenic mycotoxins when eating moldy garbage, such as dairy products, nuts, grains, bread, and spaghetti (*64*). More than 20 mycotoxins have been classified as tremorgens, with penitrem A and roquefortine being the most important ones for companion animals (*6*, *65*, *66*).

Most tremorgenic mycotoxins are neurotoxic indole alkaloids (**Figure 1**). Penitrem A, for instance, causes slight salivation, vomiting, ataxia, and tremors in dogs, possibly by inhibiting inhibitory neurotransmitters, such as glycine and γ-aminobutyric acid in the central nervous system (*6*, *64*, *67*, *68*). The mechanism of action of roquefortine, however, has not been studied in detail.

Oral exposure to 0.175 mg of penitrem A/kg of bw was sufficient to induce muscle tremors in dogs (*67*). The oral LD_{50} values for penitrem A and roquefortine are 1.1 and 20 mg/kg of bw in mice, respectively (*69*, *70*). There have been at least 10 reports of tremorgenic mycotoxicoses in dogs due to garbage consumption since 1979, mostly in North America (*64*, *67*, *68*, *71*−*78*).

3. GOVERNMENT REGULATION OF MYCOTOXINS IN PET FOOD

Regulation of mycotoxin content of animal feed worldwide mainly focuses on farm animals, with less attention to companion species. In most countries, pet food is regulated by a maximum mycotoxin contamination for all feedstuffs rather than pet-specific legislation. Both the United States and Canada, for example, have a 20 μg/kg legal limit for aflatoxins B_1, B_2, G_1, and G_2 for all animal feed (*79*, *80*; **Table 4**). The U.S. recommendations on deoxynivalenol and fumonisins also apply to all feedstuffs (*79*, *81*). The Canadian recommendations on deoxynivalenol, ochratoxin A, and zearalenone, in contrast, apply only to cattle, poultry, and swine feedstuffs (*81*). The European Union has imposed a 20 μg/kg legal limit for aflatoxin B_1 in animal feed (*82*) and issued guidance values for deoxynivalenol and fumonisin (*83*).

Mycotoxin regulations vary among different countries. Whereas at least 50 countries regulate the aflatoxin content of feedstuffs,

Table 2. Major Mycotoxin Outbreaks in Pets

year	location	scale	diet	mycotoxin level	refs
1951–1955	southeastern U.S.	71 food poisoning cases of dogs (with several dead)	a brand of commercial dog food suspected to be made with contaminated corn	unknown	*13–15*
1974	Rajasthan and Gujarat, India	reports of dead stray and domestic dogs in about 200 villages (97 people died in the same outbreak)	contaminated corn	6.25–15.6 mg of aflatoxins B_1 and G_1/kg of food sample	*166*, *167*
1974	Alabama (U.S.)	at least 3 dogs dead and several with anorexia and depression in a kennel after chronic mycotoxin exposure	cooked cornmeal, meat scraps, and contaminated dry commercial dog food	60 μg of aflatoxin B_1/kg of feed	20
1975	Queensland, Australia	3 dogs dead after acute and subacute mycotoxin exposure	mixture of bread loaves (moldy) and canned dog meat	vomit samples contained 100 and 40 mg of aflatoxins B_1 and G_1/kg, respectively	21
1985	Georgia (U.S.)	13 deaths and several with weakness, icterus, and partial anorexia of 48 dogs in a kennel	mixture of commercial dog food and restaurant garbage	unknown	*22*
1986	Georgia (U.S.)	9 deaths of 20 dogs in a kennel	ration of contaminated cornmeal, soybean meal, fat, and a mineral supplement	465 and 46 μg of aflatoxins B_1 and B_2/kg of feed, respectively	*22*
1987	Germany	6 puppies dead	a brand of contaminated commercial milk powder for puppies	6.8 μg of ochratoxin A/kg of powder (herpesvirus infection was also involved)	40
1987	Pretoria, South Africa	10 dogs dead with 1 acute, 7 subacute, and 2 chronic cases	a brand of contaminated commercial dog food	100–300 μg of aflatoxins/kg of feed	16
1988	Australia	1 dog with severe muscle tremors	moldy hamburger bun	35 mg of penitrem A/kg of sample	*67*
1991	Austria	3 mycotoxicosis cases involving pet birds (a–c; all birds had recovered)	(a) rearing feed; (b) millet seed cobs; and (c) commercial bird feed	(a) 20 μg of aflatoxin B_1/kg of feed; (b) 15 μg of aflatoxin B_1/kg of feed; and (c) 2 mg of nivalenol, 0.3 mg of deoxynivalenol, and 0.1 mg of diacetoxyscirpenol/kg of feed, respectively	*168*
1991	Glasgow, U.K.	1 dog with hepatopathy and dermatitis	damp and moldy biscuit meal	100 μg of ochratoxin A, 150 μg of citrinin, and 200 μg of sterigmatocystin/kg of meal	*41*
1998	Texas (U.S.)	55 dogs dead with both acute and chronic cases	17 different formulations of commercial dog food made with two rail cars of nonuniformly contaminated corn in a milling plant in Texas in late summer	100–300 μg of aflatoxin B_1/kg of feed	17
2002	Massachusetts (U.S.)	4 dogs with ataxia and tremors	garbage	unknown level of tremorgenic mycotoxins	64
2002	South Africa	2 dogs with vomiting and tremors	moldy rice	2.6 mg of penitrem A and 34 mg of roquefortine/kg of rice	68
2005	eastern U.S.	at least 100 dogs dead	19 different formulations of commercial dog food made with contaminated corn in a milling plant in South Carolina in summer	unknown	*18*, *19*
2006	Korea	3 dogs dead with renal failure	fungal nephrotoxins in the diet, possibly ochratoxin and citrinin	unknown	42

only 9, with 6 in Europe, have similar regulations for ochratoxin A (*81*). Switzerland and Brazil, for instance, have legal limits of 10 and 50 μg/kg, respectively, for aflatoxin B_1 in all animal feeds (*84*, *85*). Government regulations of mycotoxin contami-

Table 3. Mycotoxin Surveys of Commercial Pet Food

year	location	samples surveyed	detection methods and limits	mycotoxins detected	ref
1993	Iowa (U.S.A.)	2 dry dog foods, 2 dry cat foods, and 1 rat chow	HPLC with C_{18} SPE column cleanup fumonisins B_1 and B_2, 200 and 20 μg/kg, respectively	fumonisins B_1 and B_2 were detected in all five samples, ranging from 219 to 1410 μg/kg and from 20 to 144 μg/kg, respectively	*62*
1994	North Carolina (U.S.A.)	100 dry dog food samples	ELISA with TLC confirmation aflatoxin B_1, 5 μg/kg zearalenone, 100 μg/kg	no sample contained >20 μg of aflatoxin B_1/kg; one sample contained 250 μg of zearalenone/kg	*11*
1997	Slough, U.K.	100 pet food samples 35 dry dog foods 35 dry dog cat foods 15 domestic bird foods 15 wild bird foods	HPLC with immunoaffinity column cleanup aflatoxins B_1, B_2, G_1, and G_2, 1.0 μg/kg, respectively ochratoxin A, 1.0 μg/kg fumonisins: not specified	mycotoxins were detected in 16% of the samples, including 7 of 30 bird foods tested aflatoxin B_1, 2 samples with 2.1 and 370 μg/kg, respectively ochratoxin A, 10% of the samples, 1–7 μg/kg fumonisins, 30% of the samples, 90–690 (B_1) μg/kg	*23*
2001	Austria and Poland	10 dog foods (2 dry, 10 canned) and 28 cat foods (8 dry, 20 canned)	HPLC with immunoaffinity column cleanup ochratoxin A, 1.0 μg/kg	ochratoxin A was detected in 47% of the pet food samples dry food, 0.21–3.2 μg of ochratoxin A/kg (with 13.1 μg/kg for one sample) canned food, 0.22–0.8 μg of ochratoxin A/kg	*39*
2001	Mexico	19 dog foods and 16 cat foods	HPLC with immunoaffinity column cleanup aflatoxins B_1, B_2, G_1, G_2, M_1, M_2, and P_1, 3–7 μg/kg	aflatoxins were detected at 89 and 100% of the dog and cat food samples, with mean aflatoxin B_1 values of 5.00 and 8.02 μg/kg, respectively	*27*
2001	Texas (U.S.A.)	142 wild bird seed samples	ELISA total aflatoxins, 1 μg/kg	aflatoxin concentrations ranged from 0 to 2780 μg/kg 17% of the samples had aflatoxin concentrations of >100 μg/kg	*25*
2002	Alfenas, Brazil	100 pet food samples 45 dog foods 25 cat foods 30 bird foods	TLC aflatoxins B_1, B_2, G_1, and G_2, 8, 6, 7, and 5 μg/kg, respectively	aflatoxins were found in 6.7, 4.0, and 26.7% of the dog, cat, and bird food samples, with mean aflatoxin B_1 levels of 19, 16, and 110 μg/kg, respectively	*24*
2002	Turkey	18 dog food samples	ELISA with immunoaffinity column cleanup total aflatoxins, 1.75 μg/kg	aflatoxins were found in 16.7% of the samples, ranging from 1.75 to 20 μg/kg	28
2003	Austria	55 cat foods (45 dry, 10 canned)	HPLC with immunoaffinity column cleanup ochratoxin A, 0.1 μg/kg	ochratoxin A was found in 7 dry and 7 canned food samples, ranging from 0.11 to 2.17 μg/kg	*40*
2003	Portugal	60 dry pet food samples: 20 dog foods 20 cat foods 20 domestic bird foods	HPLC with immunoaffinity column cleanup aflatoxins B_1, B_2, G_1, and G_2, 1 μg/kg, respectively ochratoxin A, 2 μg/kg fumonisin B_1, 10 μg/kg deoxynivalenol, 100 μg/kg	mycotoxins were detected in only dog food aflatoxins, not detected ochratoxin A, 5 samples, 2.0–3.6 μg/kg fumonisin B_1, 3 samples, 12.0–24.0 μg/kg deoxynivalenol, 3 samples, 100.0–130.0 μg/kg	*30*
2004	Poland	57 brands of standard and therapeutic pet food	HPLC with immunoaffinity column cleanup zearalenone, 5.0 μg/kg	zearalenone was detected in 84% of the samples mean, 36.2 μg/kg highest levels, 299.5 μg/kg (standard) and 158.0 μg/kg (therapeutic)	63

nation appear to reflect analytical detection limits and regional prevalence as well as trade relationships among different countries, but do not necessarily represent the safe limit for mycotoxin exposure in pet animals.

4. CHALLENGES IN THE PET FOOD INDUSTRY

The pet food industry has the same risks regarding mycotoxin contamination of raw materials as the feed industry for agricultural species. The most common challenges in the pet food industry include grain processing, sampling error, analytical methods, synergistic interactions, storage conditions, and conjugated mycotoxins.

4.1. Grain Processing. Cereal byproducts such as bran and feed grade flour are often included in the formulation of pet food, although they may contain concentrated levels of mycotoxins compared to grits and flour for human consumption. Brera et al. (*4*, *5*) demonstrated that aflatoxins, zearalenone, and fuminosin B_1 were concentrated by at least 3-fold in bran, germ, and feed grain compared to the raw corn kernels during an industrial milling process, whereas the mycotoxin level in the grain fractions for human consumption was reduced. Fungal growth and mycotoxin contamination are more severe in the outer part of grains, contributing to the concentrating effect in the bran and germ fractions. The inclusion of bran and germ in turn causes a higher mycotoxin level in animal flour. The grain fractions intended for animals may, therefore, contain higher mycotoxin levels than raw grains.

Table 4. Regulations for Mycotoxins (Milligrams per Kilogram) in Pet Foods in Selected Regions

region	aflatoxins[a]	aflatoxin B_1	deoxynivalenol	zearalenone	fumonisins	refs
Canada	0.02[b]	NR[c]	NR	NR	NR	*80*, *81*
European Union[d]	NR	0.02[b]	5[e]	NR	5[e]	*82*, *83*
Japan	0.02[b]	NR	1[b]	1[b]	NR	*81*
United States	0.02[b]	NR	5[e]	NR	10[e,f]	*79*, *81*

[a] Total content of aflatoxinS B_1, B_2, G_1, and G_2. [b] Legal limit. [c] Not regulated in pet food. [d] Harmonized regulation in member countries; each may have additional mycotoxin regulations. [e] Guidance value. [f] 5 mg/kg for rabbit feed.

4.2.1. Challenges in Sampling. The objective of sampling raw materials is to obtain samples that truly represent the grains that are arriving in a shipment or that are in storage. Characteristics to be measured include proximate composition, moisture content, mold and insect damage, and the presence of mycotoxins.

Unlike nutritional characteristics, mycotoxins are not evenly distributed in grains. Areas of concentrated contamination are commonly found in storage bins, arising from the contamination of certain areas in the crop, the mixture of contaminated grains with uncontaminated grains, and the formation of high-moisture areas. Cucullu et al. (*86*) reported that 18% of cottonseed kernels were contaminated with aflatoxin B_1, with individual concentrations ranging from 150 to 5 750 000 μg/kg. Johnson et al. (*87*), in another study, reported aflatoxin B_1 contamination in 72 samples of corn from two bins ranging from 0 to 376 μg/kg with an average of 21 μg/kg in the first bin and from 0 to 332 μg/kg with an average of 15 μg/kg in the second bin. It has also been reported that uncontaminated kernels surround aflatoxin-contaminated kernels. Sixteen of 140 individually analyzed corn kernels were contaminated with aflatoxin B_1, ranging from 260 to 38000 μg/kg, and 12 of the 140 kernels were contaminated with zearalenone, ranging from 9 to 1700 mg/kg (*88*).

Sampling operation is often the biggest source of error in mycotoxin analysis due to uneven distribution of mycotoxins in grains (*89*). Miraglia et al. (*90*) purposed that an ideal sampling protocol should include (1) objective-orientated analysis to determine why, where, and when to obtain samples and (2) a sampling method to take into account the heterogeneous nature of mycotoxin contamination.

The U.S. Department of Agriculture has a test procedure consisting of a 5 kg sample, a 250 g subsample, and a single TLC analysis (*91*). The total associated variance of this procedure is 630.7, including 521.4 (82.7%) due to the sampling, 59.2 (9.4%) due to subsampling, and 50.1 (7.9%) due to the TLC analysis (*92*). Increasing the sample size to 20 kg, using a vertical cutter mixer to take a 100 g subsample instead of the USDA/Agricultural Marketing Service 250 g subsample, and replacing TLC with HPLC can reportedly reduce the total variance to 176. Distribution of variance will then be 130.4 (74.1%) due to sampling, 25.5 (14.5%) due to subsampling, and 20.1 (11.4%) due to analysis (*92*).

Feed mills usually have a maximal tolerance for a given mycotoxin in a shipment to determine acceptance. If the sample is not taken appropriately, it is possible that a shipment with higher concentrations of mycotoxins than the maximum permitted will be accepted. A shipment that truly has lower concentrations of mycotoxins may also be rejected. Reducing the variability will, therefore, reduce the risk for both the seller and the buyer of rejecting an acceptable shipment or accepting an unacceptable shipment, respectively (*89*, *91*).

4.2.2. Sample Preparation for Analysis. Variation in subsampling is less than that seen in sampling. Subsamples should be prepared by (1) coarse grinding of 1 kg of the sample to a particle size that can pass a standard 14 mesh screen; (2) mixing to homogenize material; (3) subdividing to take a portion for further fine grinding that can pass a standard 20 mesh screen; and (4) taking a subsample for analysis that can be 25−100 g (*93*). The use of a vertical cutter mixer can reduce the variance of subsampling (*91*).

4.3. Analytical Methods. There are different methods to analyze mycotoxins. To analyze aflatoxins, TLC is uncomplicated and requires less expensive equipment than other analytical methods. The lower limit of detection of aflatoxin both in corn and in cottonseed meals is 1 μg/kg with TLC (*87*). The accuracy of TLC, however, is not as high as more expensive methods such as HPLC. The limit of detection of HPLC for aflatoxins is as low as 0.3 μg/kg (*94*). To analyze zearalenone, TLC is less sensitive with a detection limit of 50 μg/kg. GC has a detection and quantification limit of 20 μg/kg, and GC-MS has a limit of 0.5 μg/kg (*95*).

The use of TLC and GC to analyze trichothecenes is limited because interfering substances can cochromatograph with toxins, thereby confounding identification (*96*). The use of HPLC is considerably more difficult because these mycotoxins do not absorb or fluoresce in UV light (*96*). A more appropriate method for trichothecene analysis is the combination of GC-MS that eliminates most of the complication related to those substances that interfere with the detection of trichothecenes (*96*). Simultaneous determination of deoxynivalenol, 3-acetyldeoxynivalenol, nivalenol, fusarenon-X, T-2 toxin, neosolaniol, diacetoxyscirpenol, and zearalenone using GC-MS has been described (*97*). This provides a convenient tool for the detection of trichothecenes and zearalenone in cereals.

Various TLC, LC, and HPLC protocols have also been developed to detect and quantify fumonisins and ochratoxin A. Preis and Vargas (*98*), for instance, described a TLC protocol for fumonisin B_1 with a detection limit of 0.1 mg/kg. In another study, Visconti et al. (*99*) lowered the detection limit to 0.05 mg/kg using LC. Martins et al. (*100*) developed a HPLC method for ochratoxin A with a detection limit of 0.2 mg/kg.

Further development of purification protocols, chromatographic methods, and detection techniques may further reduce the detection limit of different mycotoxins. Ventura et al. (*101*), for instance, described a detection method to detect as little as 0.1 μg/L of aflatoxins and ochratoxin A simultaneously using ultraperformance liquid chromatography−tandem MS and a solid-phase extraction. The present trend in chromatographic mycotoxin analysis is to use immunoaffinity columns (IAC) as a sample purification technique to improve sensitivity, although there are concerns that IAC may affect the accuracy of analysis (*102*).

Commercial enzyme-linked immunosorbent assay (ELISA) kits are simple, cheap, rapid, and widely adopted as in-house screening methods for individual mycotoxins (*103*). Direct competitive ELISA is most often used in mycotoxin analysis (*104*). ELISA may produce false-positive results, however, due to antigen−antibody cross-reactions, so further confirmation

using HPLC or GLC is sometimes necessary (*103*). Recent development of molecular imprint materials and recombinant antibodies for specific mycotoxins promises novel applications in ELISA and sample purification (*105*, *106*).

4.4. Conjugated Mycotoxins. The possibility of conjugated mycotoxins in plants has been suggested. Savart (*107*) was the first to synthesize in vitro eight fatty acid esters and two glucoside conjugates of deoxynivalenol. In another study, detection of deoxynivalenol-3-glucoside was reported in both naturally and artificially contaminated wheat and corn, suggesting that plants can transform deoxynivalenol into conjugated forms (*108*). Detection of conjugated mycotoxins is difficult because these are more polar compounds than the precursor toxins, and the extraction of conjugated mycotoxins with conventional solvents is more difficult due to losses in the sample purification process (*108*).

The natural occurrence of conjugated zearalenone, such as zearalenone-4-β-D-glucopyranoside, has been reported in both corn and wheat (*109*). In the same study, the authors found that the conjugated zearalenone was present in 42% of contaminated wheat samples. It was concluded that metabolic processes in the wheat cell produce the glucopyranoside conjugate of zearalenone.

Fumonisins have been found to react with the food matrix in such a way that the amino group essential for detection by derivatization is blocked (*110*). Shier et al. (*111*) showed that fumonisins may bind to protein in roasted corn meal dough and escape HPLC detection. Seefelder et al. (*112*) suggested that fumonisins could conjugate to proteins as well as to polysaccharides via the free tricarballylic acid side chains.

Conjugated forms of ochratoxin A have been discovered in fungal culture (*113*). There is no information, however, on the natural occurrence of conjugated ochratoxin A in grains or flour.

4.5. Storage Conditions. Conditions of grain storage are very important to prevent mycotoxin production and mold spoilage. The most important physical factors involved in the production of mycotoxins are temperature, water activity or moisture content, and atmospheric composition (*114*). In stored cereals, a moisture content of >15% is required to maintain fungal viability (*115*). There is an upper limit of moisture content for the stable storage of grains, which may be close to the moisture content of grains at harvest. When crops are harvested with moisture content in excess of the safe level, grains must be dried immediately (*115*). There are two types of transfer of heat and/or moisture in stored grains: (1) forced cold drying and (2) forced hot drying and aeration.

Transfer of water vapor and heat occurs mainly between the grains and the air that circulates around the grains. The air and grains, therefore, tend toward a hydric and thermal equilibrium (*116*). Natural transfer occurring in storage in the absence of forced air is uncontrolled and unpredictable. Differences in moisture content result in the transfer of moisture from humid to dry grains when dry grains are mixed with wet grains. More than 1 week is required to reach the moisture equilibrium. When grains of different moisture levels are stored together without mixing, the time to accomplish moisture equilibrium is longer (*116*).

The formation of wet spots results from moisture transfer when grains of different temperatures are contained in the same bin. It is common to find areas of cold grain and areas of warm grain in the same bin, and air moves from warm grains to cold grains. When warm air passes through cold grains, air moisture condenses and the moisture content of the cold grains increases (*117*).

The so-called "cold wall" effect introduces temperature gradients to stored grains. This effect is caused by diurnal and seasonal variations of grain temperature. One side of a bin may be exposed to sunlight with the other side in the shade, resulting in a temperature difference. During transport by boat, there can be differences between the sides of the ship that are in contact with cold water and in the internal part of silos that are warmer. Heat and moisture are transferred from the hottest to the coldest grains, and dry grains are moistened. When entering air currents have a higher dew point than the grains through which they are circulating, condensation of water occurs on the surface of grains and the water is absorbed (*116*). Good storage can prevent mold growth and mycotoxin production. It is clear that storing grains with moisture content below 15% or water activity below 0.7 prevents the formation of mycotoxins in stored grains (*118*). Good management practices for stored grains, therefore, reduce the risk of mycotoxin production.

4.6. Synergistic Interactions among Mycotoxins. It is known that synergistic interactions among mycotoxins occur in some domestic species. A toxicological synergism between deoxynivalenol and fusaric acid has been demonstrated in 8 kg piglets, where deoxynivalenol toxicity was augmented when fusaric acid was added in the diet (*119*). In another study, it was found that chickens fed combinations of deoxynivalenol and T-2 toxin from hatching to 3 weeks had significantly reduced body weight gain (*120*). This variable was not reduced, however, when either deoxynivalenol or T-2 toxin was fed singly, thereby suggesting a synergistic interaction.

Aflatoxins, ochratoxins, and other mycotoxins have also been demonstrated to interact synergistically. The feeding of diets containing both aflatoxins and ochratoxin A to chickens from hatching to 3 weeks of age resulted in significantly greater relative weight of gizzard and kidneys as well as less weight gain compared to either mycotoxin fed singly (*121*). Citrinin and penicillic acid were also found to potentiate the nephrotoxic and carcinogenic effects of ochratoxin A, resecptively (*122*, *123*).

5. PREVENTATIVE STRATEGIES

Mycotoxins are, in general, chemically and thermally stable compounds. Once a mycotoxin-contaminated ingredient is screened and enters the milling process, mycotoxins are likely to be retained in the finished product, and further removal of mycotoxins is practically impossible. Most preventative strategies, therefore, focus on removing contamination early in processing or preventing mycotoxicoses in vivo without compromising nutritional quality.

5.1. Processing Techniques. There are three major processing techniques to reduce mycotoxin content of cereals: sieving, washing, and pearling. Cracked, damaged, and improperly developed kernels are good substances for fungal growth. Most mycotoxins are found at high concentration in this fraction as well as in dust and debris formed during grain handling. The separation of these more contaminated fractions from kernels may markedly reduce the mycotoxin content of cereal grains. Trenholm et al. (*124*) investigated the efficacy of sieving on corn, barley, and wheat contaminated with 5−23 mg of deoxynivalenol/kg and 0.5−1.21 mg of zearalenone/kg. After being sieved with a series of screens, the retained fractions contained 67−83% less mycotoxins with a 34−69% material loss of the original kernel.

Washing methods, on the other hand, take advantage of the fact that mycotoxins are primarily found on the outer surface of grains. Treholm et al. (*125*) were the first to report a washing

technique to decontaminate grains containing deoxynivalenol and zearalenone. After a first wash with 1 M sodium carbonate solution and two extra washes with distilled water, barley and corn showed 72−74 and 80−87% reductions in deoxynivalenol and zearalenone contents, respectively. In another study, a 1-day hot water (80 °C) treatment with 1 M sodium carbonate reduced the deoxynivalenol content of barley from 18.4 to 1.4 mg/kg (*126*). A major disadvantage of washing methods, however, is that the wetted cereal grains need drying, causing an extra cost to feed manufacturers.

House et al. (*127*) developed an abrasive pearling procedure that removed 66% of deoxynivalenol content with a 15% material loss of barley in 15 s without wetting the cereal. Siwela et al. (*128*) reported that the aflatoxin content of corn was reduced by 93.4% after a physical dehulling procedure.

Ozonation has also been proposed as a detoxifying method for mycotoxin-contaminated grains. Procotor et al. (*129*) evaluated the effectiveness of ozonation in breaking down aflatoxins in peanut kernels and flour. Up to 60% of aflatoxin B_1 was degraded after a 15-min gaseous ozonation treatment at room temperature. An aqueous treatment of 25 mg/mL ozone was found to destroy the epoxy group of trichothecenes, although the toxicity of residual compounds required further investigation (*130*).

Acid-based mold inhibitors are commonly used by the animal industry to prevent mycotoxin formation in animal feed. Weak organic acids including benzoic, acetic, sorbic, and propionic acids are known to inhibit fungal growth by acidifying the cytoplasmic content of fungal cells (*131*, *132*). In addition to applying these processing techniques, it is also important to purchase high-quality feedstuffs and to handle the grain carefully to prevent kernel damage, thereby minimizing mycotoxin formation.

5.2. Nutrient Supplementation. A number of nutrient supplements have been proposed to act therapeutically to minimize mycotoxin-induced tissue damage and altered behavior. These nutrients include large neutral amino acids, antioxidants, and polyunsaturated fatty acids (PUFA).

Supplemental amino acids primarily alleviate the effects of mycotoxins on the central nervous system. Fusaric acid, T-2 toxin, and deoxynivalenol elevate blood and brain tryptophan concentrations, increase brain serotonin concentrations, and thereby result in feed refusal and vomiting (*133*, *134*). It is possible, therefore, to supplement feedstuffs with large neutral amino acids, such as leucine, isoleucine, valine, tyrosine, and phenylalanine, to compete with tryptophan for active transporter carriers across the blood−brain barrier, thereby preventing the mycotoxin-induced brain uptake of tryptophan and subsequent overt toxicity (*135*).

Because a number of mycotoxins including aflatoxin B_1, fumonisin B_1, deoxynivalenol, T-2 toxin, and ochratoxin A are known to damage cell membranes by lipid peroxidation, the therapeutic properties of antioxidant nutrients against mycotoxins have been investigated (*136*). Selenium and vitamins A, C, and E have been demonstrated to reduce the toxic effects of mycotoxins in vitro and in vivo (*137*−*141*).

The effectiveness of antioxidants in the prevention of mycotoxin-induced toxicity has also been investigated. A few reports have also suggested that antioxidants can prevent aflatoxin-induced carcinogenicity (*142*−*144*). Pretreatments with vitamins A, C, and E in mice and rats were found to reduce DNA adduct formation in the kidney and liver caused by ochratoxin A and zearalenone (*145*). Antioxidants appear to act as superoxide anion scavengers and thereby protect cell membranes and DNA from mycotoxin-induced damage (*136*).

Omega-3 PUFA have been suggested to reduce IgA-induced nephropathy. Shi and Pestka (*146*) demonstrated that dietary supplementation with 3% (w/w) eicosapentaenoic acid (EPA) suppressed the development of IgA nephropathy in mice consuming 20 mg of deoxynivalenol/kg of feed. In another study, docosahexaenoic acid (DHA) and EPA, but not α-linolenic acid, attenuated the proinflammatory response and renal damage induced by 10 mg of deoxynivalenol/kg of feed (*147*). It is believed that DHA and EPA reduce deoxynivalenol-induced interleukin-6 production and therefore alleviate the inflammatory damage to the kidney (*148*, *149*).

Despite the experimental findings of the protective effects of nutrient supplementation against mycotoxins, there are limited clinical feeding trials to verify their efficiency in treating mycotoxicosis in domestic and pet animals. The feasibility of using these dietary supplements to treat mycotoxicosis in pet animals, therefore, remains to be confirmed.

5.3. Mycotoxin-Sequestering Agents. Mycotoxin-sequestering agents, such as activated charcoal, silicate minerals, and cholestyramine, can prevent intestinal mycotoxin absorption in animals (*150*). Hydrated sodium calcium aluminosilicate (HSCAS) has been shown to significantly reduce the adverse effects of feeding 7.5 mg of aflatoxin B_1/kg of feed on broiler chickens with an inclusion level of 0.5% (w/w) (*161*). Further experiments using 3.5 mg of aflatoxins/kg of feed, combined with 2.0 mg of ochratoxin A/kg of feed and 8.0 mg of T-2 toxin/kg of feed, however, showed that the 0.5% HSCAS inclusion could diminish many of the adverse effects of dietary aflatoxins but had no effect on ochratoxin A or T-2 toxicity in the chickens (*152*).

Most mycotoxin-sequestering agents either only adsorb specific mycotoxins, require a high inclusion rate in animal feed, cause other health complications, or are too expensive for industrial applications. Naturally occurring glucomannan-containing polymers extracted from yeast cell wall, in contrast, have a high adsorptive capacity for binding a combination of different mycotoxins and can prevent mycotoxicoses with a low inclusion rate, thereby showing great promise as a practical solution for the pet food industry.

The efficacy of glucomannan-containing polymers as mycotoxin-sequestering agents in feeds has been investigated in several studies with domestic animals. Raju and Devegowda (*153*) reported that the feeding of 1 g of a polymeric glucomannan mycotoxin absorbent (GMA)/kg of feed significantly improved feed intake, body weight gain, and biochemical and hematological parameters of broiler chickens during in a 35-day exposure to aflatoxin B_1 (0.3 mg/kg of feed), ochratoxin A (2 mg/kg of feed), and T-2 toxin (3 mg/kg of feed). In another study, Swamy et al. (*164*) demonstrated that 0.2 g of GMA/kg of feed could prevent the changes in neurochemistry and serum antibody concentrations caused by a dietary combination of deoxynivalenol, 15-acetyldeoxynivalenol, fusaric acid, and zearalenone (5.5, 0.5, 26.8, and 0.4 mg/kg of feed, respectively) in immature swine. Similar benefits have been seen in a multiple fusarial challenge to broiler chicken (*155*), laying hens (*156*), turkeys (*157*), and horses (*158*).

5.4. Microbial Deactivation of Mycotoxins. The application of microorganisms capable of detoxifying mycotoxins into nontoxic metabolites in animal feed has been proposed to prevent mycotoxicosis (*159*). These microorganisms act in the intestinal tract of animals prior to mycotoxin absorption. A strain of bacteria (BBSH 797), for instance, was isolated from bovine

rumen fluid and found to deactivate trichothecenes by reducing epoxide rings (*160*, *161*). *Trichosporon mycotoxinivorans* (Trichosporon MTV, 115), a novel yeast strain, was found to be capable of degrading ochratoxin A and zearalenone (*159*). The detoxifying effectiveness of these strains was demonstrated in yeast and gut tissue cultures (*159*, *162*, *163*).

6. CONCLUSIONS

It has been shown that mycotoxins have various pathological effects on pet species. The worldwide prevalence of mycotoxins in pet food has also been well-documented to cause mortality and economic losses due to acute mycotoxicosis in extreme cases. Specific mycotoxin regulations on pet food should, therefore, be put in force to address the problem. Further research to develop more accurate sampling techniques, more efficient decontaminating processes, and more effective dietary supplements would protect companion animals from the harmful effects of mycotoxins until mycotoxins can be completely eliminated from the raw materials used in pet foods.

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LITERATURE CITED

(1) Haschek, W. M.; Voss, K. A.; Beasley, V. R. Selected mycotoxins affecting animal and human health. In *Handbook of Toxicologic Pathology*, 2nd ed.; Haschek, W. M., Roussex, C. G., Wallig, M. A., Eds.; Academic Press: New York, 2002; pp 645−698.

(2) Moss, M. O. Mycotoxic fungi. In *Microbial Food Poisoning*, 2nd ed.; Eley, A. R., Ed.; Chapman and Hall: New York, 1996; pp 75−93.

(3) Food and Agriculture Organization of the United Nation/International Atomic Energy Agency (FAO/IAEA). Manual on the application of the HACCP system in mycotoxin prevention and control. In *Food and Nutrition Paper*; Food and Nutrition Division, FAO: Rome, Italy, 2001; Vol. 73, pp 7−13.

(4) Brera, C.; Catano, C.; de Santis, B.; Debegnach, F.; de Giacomo, M.; Pannunzi, E.; Miraglia, M. Effect of industrial processing on the distribution of aflatoxins and zearalenone in corn-milling fractions. *J. Agric. Food Chem.* **2006**, *54*, 5014−5019.

(5) Brera, C.; Debegnach, F.; Grossi, S.; Miraglia, M. Effect of industrial processing on the distribution of fumonisin B_1 in dry milling corn fractions. *J. Food Prot.* **2004**, *67*, 1261−1266.

(6) Puschner, B. Mycotoxins. *Vet. Clin. North Am. Small Anim. Pract.* **2002**, *32*, 409−419.

(7) Böhm, J.; Razzai-Fazeli, E. Effects of mycotoxins on domestic pet species. In *The Mycotoxin Blue Book*; Diaz, D., Ed.; Nottingham University Press: Nottingham, U.K., 2005; pp 77−91.

(8) Rumbeiha, W. Mycotoxins may cause clinical symptoms in pets. *Feedstuffs* **2002**, *April 29*, 14−15.

(9) International Agency for Research on Cancer−World Health Organization (IARC-WHO). Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. In *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*; IARC Working Group, World Health Organization: Lyon, France, 1993; Vol. 56, pp 467−488.

(10) Chu, F. S. Mycotoxins. In *Foodborne Diseases*, 2nd ed.; Cliver, D. O., Riemann, H., Eds.; Academic Press: San Diego, CA, 2002; pp 271−303.

(11) Cullen, J. M.; Newberne, P. M. Acute hepatotoxicity of aflatoxins. In *The Toxicology of Aflatoxins: Human Health, Veterinary and Agricultural Significance*; Eaton, D. L., Groopman, J. D., Eds.; Academic Press: San Diego, CA, 1994; pp 3−26.

(12) Newberne, P. M.; Wogan, G. N. Sequential morphologic changes in aflatoxin B carcinogenesis in the rat. *Cancer Res.* **1968**, *28*, 700−781.

(13) Bailey, W. S.; Groth, A. H. The relationship of hepatitis X in dogs and moldy corn poisoning of swine. *J. Am. Vet. Med. Assoc.* **1959**, *134*, 483−486.

(14) Newberne, J. W.; Bailey, W. S.; Seibold, H. R. Notes on a recent outbreak and experimental reproduction of hepatitis X in dogs. *J. Am. Vet. Med. Assoc.* **1955**, *127*, 59−62.

(15) Seibold, H. R.; Bailey, W. S. An epizootic of hepatitis in the dogs. *J. Am. Vet. Med. Assoc.* **1952**, *121*, 201−206.

(16) Bastianello, S. S.; Nesbit, J. W.; Williams, M. C.; Lange, A. L. Pathological findings in a natural outbreak of aflatoxicosis in dogs. *Onderstepoort J. Vet. Res.* **1987**, *54*, 635−640.

(17) Garland, T.; Reagor, J. Chronic canine aflatoxicosis and management of an epidemic. In *Mycotoxins and Phycotoxins in Perspective at the Turn of the Millennium*; deKoe, W., Samson, R., van Egmond, H., Gilbert, J., Sabino, M., Eds.; Ponsen and Looven: Wageningen, The Netherlands, 2001; pp 231−236.

(18) U.S. Food and Drug Administration (FDA). *FDA Statement: FDA Issues Consumer Alert on Contaminated Pet Food*; Washington, DC, Dec 30, 2005.

(19) Stenske, K. A.; Smith, J. R.; Newman, S. J.; Newman, L. B.; Kirk, C. A. Aflatoxicosis in dogs and dealing with suspected contaminated commercial foods. *J. Am. Vet. Med. Assoc.* **2006**, *228*, 1686−1691.

(20) Greene, C. E.; Barsanti, J. A.; Jones, B. D. Disseminated intravascular coagulation complicating aflatoxicosis in dogs. *Cornell Vet.* **1977**, *67*, 29−69.

(21) Ketterer, P. J.; Williams, E. S.; Blaney, B. J.; Connole, M. D. Canine aflatoxicosis. *Aust. Vet. J.* **1975**, *51*, 355−357.

(22) Liggett, A. D.; Colvin, B. M.; Beaver, R. W.; Wilson, D. M. Canine aflatoxicosis: a continuing problem. *Vet. Hum. Toxicol.* **1986**, *28*, 428−430.

(23) Scudamore, K. A.; Hetmanski, M. T.; Nawaz, S.; Naylor, J.; Rainbird, S. Determination of mycotoxins in pet foods sold for domestic pets and wild birds using linked-column immunoassay clean-up and HPLC. *Food Addit. Contam.* **1997**, *14*, 175−186.

(24) Maia, P. P.; Pereira Bastos de Siqueira, M. E. Occurrence of aflatoxins B_1, B_2, G_1 and G_2 in some Brazilian pet foods. *Food Addit. Contam.* **2002**, *19*, 1180−1183.

(25) Henke, S. E.; Gallardo, V. C.; Martinez, B.; Balley, R. Survey of aflatoxin concentrations in wild bird seed purchased in Texas. *J. Wildlife Dis.* **2001**, *37*, 831−835.

(26) Cullen, J. M.; Hagler, W. M. Survey of commercial dog food for aflatoxin B_1 and zearalenone. In *Biodeterioration Research 4*; Llewellyn, G. C., Ed.; Plenum Press: New York, 1994; pp 79−83.

(27) Sharma, M.; Marquez, C. Determination of aflatoxins in domestic pet foods (dog and cat) using immunoaffinity column and HPLC. *Anim. Feed Sci. Technol.* **2001**, *93*, 109−114.

(28) Gunsen, U.; Yaroglu, T. Aflatoxin in dog and horse feeds in Turkey. *Vet. Hum. Toxicol.* **2002**, *44*, 113−114.

(29) Bueno, D. J.; Silva, J. O.; Oliver, G. Mycoflora in commercial pet foods. *J. Food Prot.* **2001**, *64*, 741−743.

(30) Martins, M. L.; Martins, H. M.; Bernardo, F. Fungal flora and mycotoxins detection in commercial pet food. *Rev. Port. Cienc. Vet.* **2003**, *98*, 179−183.

(31) Council for Agricultural Science and Technology (CAST). Mycotoxins: risk in plant and animal systems. In *Task Force Report 139*; CAST: Ames, IA, 2003; p 199.

(32) Petzinger, E.; Ziegler, K. Ochratoxin A from a toxicological perspective. *J. Vet. Pharmacol. Ther.* **2000**, *23*, 91−98.

(33) Gekle, M.; Sauvant, C.; Schwerdt, G. Ochratoxin A at nanomolar concentrations: a signal modulator in renal cells. *Mol. Nutr. Food Res.* **2005**, *49*, 118−130.

(34) Szczech, G. M.; Carlton, W. W.; Tuite, J. Ochratoxicosis in beagle dogs. I. Clinical and clinicopathological features. *Vet. Pathol.* **1973**, *10*, 135−154.

(35) Stoev, S. D.; Paskalev, M.; MacDonald, S.; Mantle, P. G. Experimental one year ochratoxin A toxicosis in pigs. *Exp. Toxicol. Pathol.* **2002**, *53*, 481−487.

(36) Manderville, R. A. A case for the genotoxicity of ochratoxin A by bioactivation and covalent DNA adduction. *Chem. Res. Toxicol.* **2005**, *18*, 1091−1097.

(37) Pfohl-Leszkowicz, A.; Petkova-Bocharova, T.; Chernozemsky, I. N.; Castegnaro, M. Balkan endemic nephropathy and associated urinary tract tumours: a review on aetiological causes and the potential role of mycotoxins. *Food Addit. Contam.* **2002**, *19*, 282−302.

(38) Razzazi, E.; Böhm, J.; Grajewski, J.; Szczepaniak, K.; Kubber-Heiss, A. J.; Iben, C. H. Residues of ochratoxin A in pet foods, canine and feline kidneys. *J. Anim. Physiol. Anim. Nutr.* **2001**, *85*, 212−216.

(39) Pühringer, S.; Razzazi, E.; Kübber, A.; Iben, C. Occurrence of ochratoxin A in commercial cat food and feline kidneys. In *Proceedings to the 7th Conference of the European Society of Veterinary and Comparative Nutrition*; Druck, C., Ed.; 2003; p 37.

(40) Gareis, M.; Reubel, G.; Kröning, T.; Porzig, R. Ein Fall von infektiösem Welpensterben bei Afaghanen in Verbindüng mit der Verfutterung von Ochratoxin A - haltigem Milchpulver. *Tieraerztl. Umsch.* **1987**, *42*, 77−80.

(41) Little, C. J. L.; McNeil, P. E.; Robb, J. Hepatopathy and dermatitis in a dog associated with the ingestion of mycotoxins. *J. Small Anim. Pract.* **1991**, *32*, 23−26.

(42) Jeong, W. I.; Do, S. H.; Jeong, D. H.; Chung, J. Y.; Yang, H. J.; Yuan, D. W.; Hong, I. H.; Park, J. K.; Goo, M. J.; Jeong, K. S. Canine renal failure syndrome in three dogs. *J. Vet. Sci.* **2006**, *7*, 299−301.

(43) Placinta, C. M.; D'Mello, J. P. F.; MacDonald, A. M. C. A review of worldwide contamination of cereal grains and animal feeds with *Fusarium* mycotoxins. *Anim. Feed Sci. Technol.* **1999**, *78*, 21−37.

(44) Desjardins, A. E. *Fusarium Mycotoxins: Chemistry, Genetics, and Biology*; APS Press: St. Paul, MN, 2006; pp 13−108, 118−119.

(45) Doko, M. B.; Canet, C.; Brown, N.; Sydenham, E. W.; Mpuchane, S.; Siame, B. A. Natural co-occurrence of fumonisins and zearalenone in cereals and cereal-based foods from eastern and southern Africa. *J. Agric. Food Chem.* **1996**, *44*, 3240−3243.

(46) Doko, M. B.; Rapior, S.; Visconti, A.; Schjoth, J. E. Incidence and levels of fumonisin contamination in maize genotypes grown in Europe and Africa. *J. Agric. Food Chem.* **1995**, *43*, 429−434.

(47) Chulze, S. N.; Ramirez, M. L.; Farnochi, M. C.; Pascale, M.; Visconti, A.; March, G. *Fusarium* and fumonisin occurrence in Argentinian corn at different ear maturity stages. *J. Agric. Food Chem.* **1996**, *44*, 2797−2801.

(48) Trucksess, M. W.; Stack, M. E.; Allen, S.; Barrion, N. Immunoaffinity column coupled with liquid chromatography for determination of fumonisin B_1 in canned and frozen sweet corn. *J. AOAC Int.* **1995**, *78*, 705−710.

(49) Yamashita, A.; Yoshizawa, T.; Aiura, Y.; Sanchez, P. C.; Dizon, E. I.; Arim, R. H.; Sardjono, B. D. *Fusarium* mycotoxins (fumonisins, nivalenol and zearalenone) and aflatoxins in corn from southeast Asia. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 1804−1807.

(50) Trucksess, M. W.; Thomas, F.; Young, K.; Stack, M. E.; Fulgueras, W. J.; Page, S. W. Survey of deoxynivalenol in U.S. 1993 wheat and barley crops by enzyme-linked immunosorbent assay. *J. AOAC Int.* **1995**, *78*, 631−636.

(51) Scott, P. M. Multi-year monitoring of Canadian grains and grain-based foods for trichothecenes and zearalenone. *Food Addit. Contam.* **1997**, *14*, 333−339.

(52) Hughes, D. M.; Gahl, M. J.; Graham, C. H.; Grieb, S. L. Overt signs of toxicity to dogs and cats of dietary deoxynivalenol. *J. Anim. Sci.* **1999**, *77*, 693−700.

(53) Kuiper-Goodman, T.; Scott, P. M.; Watanabe, H. Risk assessment of the mycotoxin zearalenone. *Regul. Toxicol. Pharmacol.* **1987**, *7*, 253−306.

(54) Gajecka, M.; Jakimiuk, E.; Polak, M.; Otrocka-Domagala, I.; Janowski, T.; Zwierzchowski, W.; Obremski, K.; Zielonka, L.; Apoznanski, J.; Gajecki, M. Zearalenone applied per os provides adverse effects in structure of chosen parts of bitch reproductive system. *Pol. J. Vet. Sci.* **2004**, *7*, 59−66.

(55) Gajecka, M.; Jakimiuk, E.; Skorska-Wyszynska, E.; Zielonka, L.; Polak, M.; Paluszewski, A.; Rybarczyk, L.; Gajecki, M. Influence of zearalenone mycotoxicosis on selected immunological, haematological and biochemical indexes of blood plasma in bitches. *Pol. J. Vet. Sci.* **2004**, *7*, 175−180.

(56) International Agency for Research on Cancer−World Health Organization (IARC-WHO). Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. In *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*; IARC Working Group, World Health Organization: Lyon, France, 2002; Vol. 82, p 301.

(57) Battista, A. F.; Flamm, E. S.; Goldstein, M.; Freedman, L. S. Effect of dopamine-β-hydroxylase inhibition on cerebral vasospasm in the cat. *J. Neurosurg.* **1976**, *44*, 168−172.

(58) Ishii, Y.; Fujii, Y.; Mimura, C.; Umezawa, H. Pharmacological action of FD-008, a new dopamine β-hydroxylase inhibitor. I. Effects on blood pressure in rats and dogs. *Arzneimittelforschung* **1975**, *25*, 55−59.

(59) Nagatsu, T.; Hidaka, H.; Kuzuya, H.; Takeya, K.; Umezawa, H. Inhibition of dopamine β-hydroxylase by fusaric acid (5-butylpicolinic acid) in vitro and in vivo. *Biochem. Pharmacol.* **1970**, *19*, 35−44.

(60) Matsuzaki, M.; Yoshida, A.; Akutsu, S.; Tsuchida, M.; Okuyama, D. Studies on toxicity of fusaric acid-Ca. IV. Chronic toxicity in dogs. *Jpn. J. Antibiot.* **1976**, *29*, 518-542.

(61) Matsuzaki, M.; Yoshida, A.; Tsuchida, M.; Sekino, M.; Asano, M. Studies on toxicity of fusaric acid-Ca. III. Subacute toxicity. *Jpn. J. Antibiot.* **1976**, *29*, 491−517.

(62) Hopmans, E. D.; Murphy, P. A. Detection of fumonisins B_1, B_2, and B_3 and hydrolyzed fumonisin B_1 in corn-containing foods. *J. Agric. Food Chem.* **1993**, *45*, 1655−1658.

(63) Zwierzchowski, W.; Gajecki, M.; Obremski, K.; Zielonka, L.; Baranowski, M. The occurrence of zearalenone and its derivatives in standard and therapeutic feeds for companion animals. *Pol. J. Vet. Sci.* **2004**, *7*, 289−293.

(64) Boysen, S. R.; Rozanski, E. A.; Chan, D. L.; Grobe, T. L.; Fallon, M. J.; Rush, J. E. Tremorgenic mycotoxicosis in four dogs from a single household. *J. Am. Vet. Med. Assoc.* **2002**, *221*, 1441−1444.

(65) Beasley, V. R.; Dorman, D. C.; Fikes, J. D.; Diana, S. G. *A Systems Affected Approach to Veterinary Toxicology*; University of Illinois Press: Urbana, IL, 1997; pp 137−142.

(66) Cole, R. J. Tremorgenic mycotoxins. In *Mycotoxins in Human and Animal Health, Conference Proceedings*; Rodricks, J. V., Ed.; Pathotox: Park Forest, IL, 1977; pp 583−595.

(67) Hocking, A. D.; Holds, K.; Tobin, N. F. Intoxication by tremorgenic mycotoxin (penitrem A) in a dog. *Aust. Vet. J.* **1988**, *65*, 82−85.

(68) Naude, T. W.; O'Brien, O. M.; Rundberget, T.; McGregor, A. D.; Roux, C.; Flaoyen, A. Tremorgenic neuromycotoxicosis in 2 dogs ascribed to the ingestion of penitrem A and possibly roquefortine in rice contaminated with Penicillium crustosum. *J. S. Afr. Vet. Assoc.* **2002**, *73*, 211−215.

(69) Ueno, Y.; Ueno, I. Toxicology and biochemistry of mycotoxins. In *Toxicology, Biochemistry and Pathology of Mycotoxins*; Uraguchi, K., Yamazaki, M., Eds.; Wiley: New York, 1988; pp 107−188.

(70) Boysen, M. E.; Jacobsson, K. G.; Schnurer, J. Molecular identification of species from the *Penicillium roqueforti* group associated with spoiled animal feed. *Appl. Environ. Microbiol.* **2000**, *66*, 1523−1526.
(71) Arp, L. H.; Richard, J. L. Intoxication of dogs with the mycotoxin penitrem A. *J. Am. Vet. Med. Assoc.* **1979**, *175*, 565−566.
(72) Richard, J. L.; Arp, L. H. Natural occurrence of the mycotoxin penitrem A in moldy cream cheese. *Mycopathologia* **1979**, *67*, 107−109.
(73) Richard, J. L.; Bacchetti, P.; Arp, L. H. Moldy walnut toxicosis in a dog, caused by the mycotoxin, penitrem A. *Mycopathologia* **1981**, *76*, 55−58.
(74) Puls, R.; Ladyman, E. Roquefortine toxicity in a dog. *Can. Vet. J.* **1988**, *29*, 569.
(75) Lowes, N. R.; Smith, R. A.; Beck, B. E. Roquefortine in the stomach contents of dogs suspected of strychnine poisoning in Alberta. *Can. Vet. J.* **1992**, *33*, 535−538.
(76) Wagner, S. O.; Podell, M.; Fenner, W. R. Generalized tremors in dogs: 24 cases (1984−1995). *J. Am. Vet. Med. Assoc.* **1997**, *211*, 731−735.
(77) Walter, S. L. Acute penitrem A and roquefortine poisoning in a dog. *Can. Vet. J.* **2002**, *43*, 372−374.
(78) Young, K. L.; Villar, D.; Carson, T. L.; Ierman, P. M.; Moore, R. A.; Bottoff, M. R. Tremorgenic mycotoxin intoxication with penitrem A and roquefortine in two dogs. *J. Am. Vet. Med. Assoc.* **2003**, *222*, 52−53.
(79) U.S. Food and Drug Administration (FDA). *Guidance for Industry: Fumonisin Levels in Human Foods and Animal Feeds*; Washington, DC, Nov 9, 2001.
(80) Canadian Feeds Act and Regulations. 1983, Section 19 (1) (I).
(81) Food and Agriculture Organization of the United Nations (FAO). *FAO Food and Nutrition Paper 81—Worldwide Regulations for Mycotoxins in Food and Feed in 2003*; FAO: Rome, Italy, 2004; p 180.
(82) European Union (EU). Commission Directive 2003/100/EC of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed. *Off. J. Eur. Communities* **2003**, *L 46*, 33−37.
(83) European Union (EU). Commission Recommendation 2006/576/EC of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. *Off. J. Eur. Communities* **2006**, *L 229*, 7−9.
(84) Verordnung des EVD vom 10. Juni 1999 über die Produktion und das Inverkehrbringen von Futternmitteln, Zusatzstoffen für die Tierernährung, Silierungszusätzen und Diätfuttermitteln (Futtermittelbuch-Verordnung, FMBV). *SR916. 307. 1, Artikel 15 und Artikel 17 (Anhang 10, Teil 1, Aflatoxin B1) SR916.307.1, Artikel 15 und Artikel 17 (Anhang 10, Teil 1, Aflatoxin B1*).
(85) Brazil Ministry of Agriculture. Portaria No. 07/88. *MAA Diario Of. Uniao* **1988**, *Seção I*, 21968−21974.
(86) Cucullu, A. F.; Lee, L. S.; Pons, W. A. Relationship of physical appearance of individual mold damaged cottonseed to aflatoxin content. *J. Am. Oil Chem. Soc.* **1977**, *54*, 235A−237A.
(87) Johnson, R. M.; Greenaway, W. T. Sampling stored corn for aflatoxin assay. *Cereal Sci. Today* **1969**, *14*, 25−29.
(88) Shotwell, O. L.; Goulden, M. L.; Bothast, R. J.; Hesseltine, C. W. Mycotoxins in hot spots in grains. I. Aflatoxin and zearalenone occurrence in stored corn. *Cereal Chem.* **1975**, *52*, 687−697.
(89) Blanc, M. Sampling: the weak link in the sanitary quality control system of agricultural products. *Mol. Nutr. Food Res.* **2006**, *50*, 473−479.
(90) Miraglia, M.; De Santis, B.; Minardi, V.; Debegnach, F.; Brera, C. The role of sampling in mycotoxin contamination: an holistic view. *Food Addit. Contam.* **2005**, *22* (Suppl. 1), 31−36.
(91) Whitaker, T. B. Standardization of mycotoxin sampling procedures: an urgent necessity. *Food Control* **2003**, *14*, 233−237.
(92) Whitaker, T. B.; Dickens, J. W.; Monroe, R. J. Variability of aflatoxin test results. *J. Am. Oil Chem. Soc.* **1974**, *51*, 214−218.
(93) Campbell, A. D.; Whitaker, T. B.; Pohland, A. E. Sampling, sample preparation, and sampling plans for foodstuffs for mycotoxin analysis. *Pure Appl. Chem.* **1986**, *58*, 305−314.
(94) Pons, W. A., Jr.; Franz, A. O. J. High pressure liquid chromatographic determination of aflatoxins in peanut products. *J. Assoc. Off. Anal. Chem.* **1978**, *61*, 793−800.
(95) Mirocha, C. J.; Schauerhamer, B.; Pathre, S. V. Isolation, detection, and quantitation of zearalenone in maize and barley. *J. Assoc. Off. Anal. Chem.* **1974**, *57*, 1104−1110.
(96) Mirocha, C. J.; Christensen, C. M. Mycotoxins. In *Storage of Cereal Grains and their Products*; Christensen, C. M., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1982; pp 241−280.
(97) Tanaka, T.; Yoneda, A.; Inoue, S.; Sugiura, Y.; Ueno, Y. Simultaneous determination of trichothecene mycotoxins and zearalenone in cereals by gas chromatography−mass spectrometry. *J. Chromatogr. A* **2000**, *882*, 23−28.
(98) Preis, R. A.; Vargas, E. A. A method for determining fumonisin B_1 in corn using immunoaffinity column clean-up and thin layer chromatography/densitometry. *Food Addit. Contam.* **2000**, *17*, 463−468.
(99) Visconti, A.; Solfrizzo, M.; De, Girolamo, A. Determination of fumonisins B_1 and B_2 in corn and corn flakes by liquid chromatography with immunoaffinity column cleanup: collaborative study. *J. AOAC Int.* **2001**, *84*, 1828−1837.
(100) Martins, M. L.; Martins, H. M.; Gimeno, A. Incidence of microflora and of ochratoxin A in green coffee beans (*Coffea arabica*). *Food Addit. Contam.* **2003**, *20*, 1127−1131.
(101) Ventura, M.; Guillen, D.; Anaya, I.; Broto-Puig, F.; Lliberia, J. L.; Agut, M.; Comellas, L. Ultra-performance liquid chromatography/tandem mass spectrometry for the simultaneous analysis of aflatoxins B_1, G_1, B_2, G_2 and ochratoxin A in beer. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 3199−3204.
(102) Castegnaro, M.; Tozlovanu, M.; Wild, C.; Molinie, A.; Sylla, A. Pfohl-Leszkowicz, A. Advantages and drawbacks of immunoaffinity columns in analysis of mycotoxins in food. *Mol. Nutr. Food Res.* **2006**, *50*, 480−487.
(103) Scudamore, K. A. Principles and applications of mycotoxin analysis. In *The Mycotoxin Blue Book*; Diaz, D., Ed.; Nottingham University Press: Nottingham, U.K., 2005; pp 157−185.
(104) Chu, F. S. Recent studies on immunoassays for mycotoxin. In *Immunoassays for Residue Analysis*; Beier, R. C., Stanker, L. H., Eds.; American Chemical Society: Washington, DC, 1996; pp 294−313.
(105) Weiss, R.; Freudenschuss, M.; Krska, R.; Mizaikoff, B. Improving methods of analysis for mycotoxins: molecularly imprinted polymers for deoxynivalenol and zearalenone. *Food Addit. Contam.* **2003**, *20*, 386−395.
(106) Yau, K. Y.; Lee, H.; Hall, J. C. Emerging trends in the synthesis and improvement of hapten-specific recombinant antibodies. *Biotechnol. Adv.* **2003**, *21*, 599−637.
(107) Savart, M. E. Deoxynivalenol fatty acid and glucoside conjugates. *J. Agric. Food Chem.* **1991**, *39*, 570−574.
(108) Berthiller, F.; Dall'Asta, C.; Schuhmacher, R.; Lemmens, M.; Adam, G.; Krska, R. Masked mycotoxins: determination of a deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography−tandem mass spectrometry. *J. Agric. Food Chem.* **2005**, *53*, 3421−3425.
(109) Schneweis, I.; Meyer, K.; Engelhardt, G.; Bauer, J. Occurrence of zearalenone-4-β-D-glucopyranoside in wheat. *J. Agric. Food Chem.* **2002**, *50*, 1736−1738.
(110) Kim, E. K.; Scott, P. M.; Lau, B. P. Hidden fumonisin in corn flakes. *Food Addit. Contam.* **2003**, *20*, 161−169.
(111) Shier, W. T.; Abbas, H. K.; Badria, F. A. Structure−activity relationships of the corn fungal toxin fumonisin B_1: implications for food safety. *Nat. Toxins* **1997**, *6*, 225−242.

(112) Seefelder, W.; Knecht, A.; Humpf, H. U. Bound fumonisin B_1: analysis of fumonisin-B_1 glyco and amino acid conjugates by liquid chromatography−electrospray ionization−tandem mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 5567−5573.

(113) Xiao, H.; Marquardt, R. R.; Abramson, D.; Frohlich, A. A. Metabolites of ochratoxins in rat urine and in a culture of *Aspergillus ochraceus*. *Appl. Environ. Microbiol.* **1996**, *62*, 648−655.

(114) Magan, N.; Hope, R.; Cairns, V.; Aldred, D. Post-harvest fungal ecology: impact of fungal growth and mycotoxin accumulation in stored grain. *Eur. J. Plant Pathol.* **2003**, *109*, 723−730.

(115) Schrödter, R. Influence of harvest and storage conditions on trichothecenes levels in various cereals. *Toxicol. Lett.* **2004**, *153*, 47−49.

(116) Multon, J. L. Spoilage mechanisms of grains and seeds in the post-harvest ecosystem, the resulting losses and strategies for the defence of stocks, In *Preservation and Storage of Grains, Seeds and their By-Products*, 1st ed.; Multon, J. L., Ed.; Lavoisier: New York, 1988; pp 3−59.

(117) Foster, G. H.; Tuite, J. Aeration and stored grain management. In *Storage of Cereal Grains and their Products*, 3rd ed.; Christensen, C. M., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1982; pp 117−143.

(118) Pelhate, J. Ecology of the microflora of grains and seeds. In *Preservation and Storage of Grains, Seeds and their By-products*, 1st ed.; Multon, J. L., Ed.; Lavoisier: New York, 1982; pp 244−262.

(119) Smith, T. K.; McMillan, E. G.; Castillo, J. B. Effect of feeding blends of *Fusarium* mycotoxin-contaminated grains containing deoxynivalenol and fusaric acid on growth and feed consumption of immature swine. *J. Anim. Sci.* **1997**, *75*, 2184−2191.

(120) Kubena, L. F.; Harvey, R. B.; Huff, W. E.; Corrier, D. E.; Phillips, T. D.; Rottinghaus, G. E. Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T-2 toxin. *Poult. Sci.* **1990**, *69*, 1078−1086.

(121) Huff, W. E.; Doer, J. A. Synergism between aflatoxin and ochratoxin A in broiler chickens. *Poult. Sci.* **1981**, *60*, 550−555.

(122) Kanisawa, M. Synergistic effect of citrinin on hepatorenal carcinogenesis of ochratoxin A in mice. *Dev. Food Sci.* **1984**, *7*, 245−254.

(123) Stoev, S. D.; Daskalov, H.; Radic, B.; Domijan, A. M.; Peraica, M. Spontaneous mycotoxic nephropathy in Bulgarian chickens with unclarified mycotoxin aetiology. *Vet. Res.* **2002**, *33*, 83−93.

(124) Trenholm, H. L.; Charmley, L. L.; Prelusky, D. B.; Warner, R. M. Two physical methods for the decontamination of four cereals contaminated with deoxynivalenol and zearalenone. *J. Agric. Food Chem.* **1991**, *39*, 356−360.

(125) Trenholm, H. L.; Charmley, L. L.; Prelusky, D. B.; Warner, R. M. Washing procedures using water or sodium carbonate solutions for the decontamination of three cereals contaminated with deoxynivalenol and zearalenone. *J. Agric. Food Chem.* **1992**, *40*, 2147−2151.

(126) Abramson, D.; House, J. D.; Nyachoti, C. M. Reduction of deoxynivalenol in barley by treatment with aqueous sodium carbonate and heat. *Mycopathologia* **2005**, *160*, 297−301.

(127) House, J. D.; Nyachoti, C. M.; Abramson, D. Deoxynivalenol removal from barley intended as swine feed through the use of an abrasive pearling procedure. *J. Agric. Food Chem.* **2003**, *51*, 5172−5175.

(128) Siwela, A. H.; Siwela, M.; Matindi, G.; Dube, S.; Nziramasanga, N. Decontamination of aflatoxin-contaminated maize by dehulling. *J. Sci. Food Agric.* **2005**, *85*, 2535−2538.

(129) Proctor, A. D.; Ahmedna, M.; Kumar, J. V.; Goktepe, I. Degradation of aflatoxins in peanut kernels/flour by gaseous ozonation and mild heat treatment. *Food Addit. Contam.* **2004**, *21*, 786−793.

(130) Young, J. C.; Zhu, H.; Zhou, T. Degradation of trichothecene mycotoxins by aqueous ozone. *Food Chem. Toxicol.* **2006**, *44*, 417−424.

(131) Hazan, R.; Levine, A.; Abeliovich, H. Benzoic acid, a weak organic acid food preservative, exerts specific effects on intracellular membrane trafficking pathways in *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* **2004**, *70*, 4449−4457.

(132) Lambert, R. J.; Stratford, M. Weak-acid preservatives: modelling microbial inhibition and response. *J. Appl. Microbiol.* **1999**, *86*, 157−164.

(133) Smith, T. K. Recent advances in the understanding of *Fusarium* trichothecene mycotoxicoses. *J. Anim. Sci.* **1992**, *70*, 3989−3993.

(134) Smith, T. K.; MacDonald, E. J. Effect of fusaric acid on brain regional neurochemistry and vomiting behavior in swine. *J. Anim. Sci.* **1991**, *69*, 2044−2049.

(135) Cavan, K. R.; MacDonald, E. J.; Smith, T. K. Potential for dietary amino acid precursors of neurotransmitters to overcome neurochemical changes in acute T-2 toxicosis in rats. *J. Nutr.* **1988**, *118*, 901−907.

(136) Atroshi, F.; Rizzo, A.; Biese, I.; Lindberg, L. A.; Saloniemi, H. Effects of feeding T-2 toxin and deoxynivalenol on DNA and GSH contents of brain and spleen of rats supplemented with vitamin E and C and selenium combination. *J. Anim. Physiol. Anim. Nutr.* **2002**, *180*, 151−167.

(137) Atroshi, F.; Biese, I.; Saloniemi, H.; Ali-Vehmas, T.; Saari, S.; Rizzo, A.; Veijalainen, P. Significance of apoptosis and its relationship to antioxidants after ochratoxin A administration in mice. *J. Pharm. Pharm. Sci.* **2000**, *3*, 281−291.

(138) Atroshi, F.; Rizzo, A.; Biese, I.; Veijalainen, P.; Antila, E.; Westermarck, T. T-2 toxin-induced DNA damage in mouse livers: the effect of pretreatment with coenzyme Q10 and α-tocopherol. *Mol. Aspects Med.* **1997**, *18*, 255−258.

(139) Atroshi, F.; Rizzo, A.; Westermarck, T.; Ali-Vehmas, T. Antioxidant nutrients and mycotoxins. *Toxicology* **2002**, *180*, 151−167.

(140) Atroshi, F.; Rizzo, A.; Westermarck, T.; Ali-Vehmas, T. Effects of tamoxifen, melatonin, coenzyme Q10, and L-carnitine supplementation on bacterial growth in the presence of mycotoxins. *Pharmacol. Res.* **1998**, *38*, 289−295.

(141) Rizzo, A. F.; Atroshi, F.; Ahotupa, M.; Sankari, S.; Elovaara, E. Protective effect of antioxidants against free radical-mediated lipid peroxidation induced by DON or T-2 toxin. *Zentralbl. Veterinarmed. A* **1994**, *41*, 81−90.

(142) Reddy, L.; Odhav, B.; Bhoola, K. Aflatoxin B_1-induced toxicity in HepG2 cells inhibited by carotenoids: morphology, apoptosis and DNA damage. *Biol. Chem.* **2006**, *387*, 87−93.

(143) Nyandieka, H. S.; Wakhisi, J. The impact of vitamins A, C, E, and selenium compound on prevention of liver cancer in rats. *East Afr. Med. J.* **1993**, *70*, 151−153.

(144) Chen, S. Y.; Chen, C. J.; Tsai, W. Y.; Ahsan, H.; Liu, T. Y.; Lin, J. T.; Santella, R. M. Associations of plasma aflatoxin B_1−albumin adduct level with plasma selenium level and genetic polymorphisms of glutathione *S*-transferase M_1 and T_1. *Nutr. Cancer* **2000**, *38*, 179−185.

(145) Grosse, Y.; Chekir-Ghedira, L.; Huc, A.; Obrecht-Pflumio, S.; Dirheimer, G.; Bacha, H.; Pfohl-Leszkowicz, A. Retinol, ascorbic acid and alpha tocopherol prevent DNA adduct formation in mice treated with the mycotoxins ochratoxin A and zearalenone. *Cancer Lett.* **1997**, *114*, 225−229.

(146) Shi, Y.; Pestka, J. J. Attenuation of mycotoxin-induced IgA nephropathy by eicosapentaenoic acid in the mouse: dose response and relation to IL-6 expression. *J. Nutr. Biochem.* **2006**, *17*, 697−706.

(147) Jia, Q.; Shi, Y.; Bennink, M. B.; Pestka, J. J. Docosahexaenoic acid and eicosapentaenoic acid, but not α-linolenic acid, suppress deoxynivalenol-induced experimental IgA nephropathy in mice. *J. Nutr.* **2004**, *134*, 1353−1361.

(148) Pestka, J. J.; Zhou, H. R.; Jia, Q.; Timmer, A. M. Dietary fish oil suppresses experimental immunoglobulin a nephropathy in mice. *J. Nutr.* **2002**, *132*, 261−269.

(149) Moon, Y.; Pestka, J. J. Deoxynivalenol-induced mitogen-activated protein kinase phosphorylation and IL-6 expression in mice suppressed by fish oil. *J. Nutr. Biochem.* **2003**, *14*, 717−726.

(150) Diaz, D. E.; Smith, T. K. Mycotoxin sequestering agents: practical tools for the neutralisation of mycotoxins. In *The Mycotoxin Blue Book*; Diaz, D., Ed.; Nottingham University Press: Nottingham, U.K., 2005; pp 323−339.
(151) Phillips, T. D.; Kubena, L. F.; Harvey, R. B.; Taylor, D. R.; Heidelbaugh, N. D. Hydrated sodium calcium aluminosilicate: a high affinity sorbent for aflatoxin. *Poult. Sci.* **1988**, *67*, 243−247.
(152) Huff, W. E.; Kubena, L. F.; Harvey, R. B.; Phillips, T. D. Efficacy of hydrated sodium calcium aluminosilicate to reduce the individual and combined toxicity of aflatoxin and ochratoxin A. *Poult. Sci.* **1992**, *71*, 64−69.
(153) Raju, M.; Devegowda, G. Influence of esterified-glucomannan on performance and organ morphology, serum biochemistry and haematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin). *Br. Poult. Sci.* **2000**, *41*, 640−650.
(154) Swamy, H. V.; Smith, T. K.; MacDonald, E. J.; Boermans, H. J.; Squires, E. J. Effects of feeding a blend of grains naturally contaminated with *Fusarium* mycotoxins on swine performance, brain regional neurochemistry, and serum chemistry and the efficacy of a polymeric glucomannan mycotoxin adsorbent. *J. Anim. Sci.* **2002**, *80*, 3257−3267.
(155) Swamy, H. V. L. N.; Smith, T. K.; Cotter, P. F.; Boermans, H. J.; Sefton, A. E. Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on production and metabolism in broilers. *Poult. Sci.* **2002**, *81*, 966−975.
(156) Chowdhury, S. R.; Smith, T. K.; Boermans, H. J.; Woodward, B. Effects of feed-borne *Fusarium* mycotoxins on hematology and immunology of laying hens. *Poult. Sci.* **2005**, *84*, 1841−1850.
(157) Chowdhury, S. R.; Smith, T. K.; Boermans, H. J.; Woodward, B. Effects of feed-borne *Fusarium* mycotoxins on hematology and immunology of turkeys. *Poult. Sci.* **2005**, *84*, 1698−1706.
(158) Raymond, S. L.; Smith, T. K.; Swamy, H. V. Effects of feeding a blend of grains naturally contaminated with *Fusarium* mycotoxins on feed intake, serum chemistry, and hematology of horses, and the efficacy of a polymeric glucomannan mycotoxin adsorbent. *J. Anim. Sci.* **2003**, *81*, 2123−2130.
(159) Schatzmayr, G.; Zehner, F.; Taubel, M.; Schatzmayr, D.; Klimitsch, A.; Loibner, A. P.; Binder, E. M. Microbiologicals for deactivating mycotoxins. *Mol. Nutr. Food Res.* **2006**, *50*, 543−551.
(160) Council for Agricultural Science and Technology (CAST). Mycotoxins: risk in plant and animal systems. In *Task Force Report 139*; CAST: Ames, IA, 2003; p 10.
(161) Binder, E. M.; Binder, J.; Ellend, N.; Schaffer, E.; Krska, R.; Braun, R. Microbiological degradation of deoxynivalenol and 3-acetyl-deoxynivalenol. In *Mycotoxins and Phycotoxins—Developments in Chemistry, Toxicology and Food Safety*; Proceedings of the IX International IUPAC Symposium on Mycotoxins and Phycotoxins; Miraglia, M., van Egmond, H., Brera, C., Gilbert, J., Eds.; Fort Collins: Alaken, CO, 1996; pp 279−285.
(162) Bruinink, A.; Rasonyi, T.; Sidler, C. Differences in neurotoxic effects of ochratoxin A, ochracin and ochratoxin-α in vitro. *Nat. Toxins* **1999**, *6*, 173−177.
(163) Schatzmayar, G.; Heidler, D.; Fuchs, E.; Mohnl, M.; Täubel, M.; Loibner, A. P.; Braun, R.; Binder, E. M. Investigation of different yeast strains for the detoxification of ochratoxin A. *Proc. Mycotoxin Res.* **2003**, *19*, 124−128.
(164) World Health Organization (WHO). *WHO Technical Report Series 906: Evaluation of Certain Mycotoxins in Food*; WHO: Geneva, Switzerland, 2002; p 62.
(165) Deshpande, S. S. Fungal toxins. In *Handbook of Food Toxicology*; Deshpande, S. S., Ed.; Dekker: New York, 2002; pp 387−457.
(166) Krishnamachari, K.; Bhat, R.; Nagarajan, V.; Tilak, T. Hepatitis due to aflatoxicosis: an outbreak in western India. *Lancet* **1975**, *305*, 1061−1063.
(167) Tandon, B. N.; Krishnamurthy, L.; Koshy, A.; Tandon, H. D.; Ramalingswami, V.; Bhandari, J. R.; Mathur, M. M.; Mathur, P. D. Study of an epidemic of jaundice, presumably due to toxic hepatitis, in northwest India. *Gastroenterology* **1977**, *72*, 488−494.
(168) Böhm, J.; Hochleithner, M. Mycotoxicosis in pet birds—selected cases. In *Proceedings to the 1st Conference of the European Committee of the Association of Avian Veterinarians*; Rubel, A., Baumgartner, R., Eds.; Association of Avian Veterinarians: Vienna, Austria, 1991; pp 255−257.

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