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ORIGINAL ARTICLE

THE PRESENCE OF DEOXYNIVALENOL AND ZEARALENONE IN INDUSTRIALLY PRODUCED DOG FEED

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

In the field of pet nutrition, the administration of complete feeds has proven to be effective. Dry complete feeds are often used for dogs, which may contain cereals among other ingredients. Cereals can be a risky ingredient due to the potential presence of mycotoxins. This work was aimed at determining the concentrations of deoxynivalenol and zearalenone in 14 samples of dry feeds for young and adult dogs obtained from different vendors. Through ELISA analysis, we found that deoxynivalenol was not found in the examined feeds in concentrations higher than the detection limit of the used kit (< 0.2 mg/kg; ppm) and zearalenone was found in 10 samples (2 samples of generic dog food, 2 samples of popular dog food and 6 samples of premium dog food) in concentrations below the detection limit of the test (< 5 µm/kg; ppb). In 4 samples of premium dog food, zearalenone was present at concentrations 21.799 ppb, 69.187 ppb, 96.745 ppb, and 163.463 ppb.

Key words: animals; feeds, mycotoxins; contamination

INTRODUCTION

The dog has always been considered man's best friend. Every responsible owner should, among other things, think about providing quality nutrition for their four-legged companion. The dog is a monogastric animal, and, like other animal species, it needs nutrients to ensure all processes take place in the body. According to the physiological effect, nutrients can be divided into building blocks (proteins and some minerals), consumables (carbohydrates and fats), and replacements (water and minerals). Due to the influence of humans and the process of domestication, dogs have become omnivores, but they share some similar nutritional features with cats, such as the lack of salivary amylase, a short gastrointestinal tract, and the inability to synthesize vitamin D [1].

Unlike cats, dogs have 3 genes (AMY2B, MGAM and SGLT1) involved in starch digestion and glucose absorption, which evolved during domestication [2]. Cats do not have these genes and are generally unable to digest fiber

efficiently [1]. Another characteristic of the canine digestive system is that they can synthesize several essential nutrients, such as niacin, taurine, and arginine [3]. As for cats, they can catabolize and use amino acids as an energy source for gluconeogenesis [4].

Feed components of animal and plant origin are used in dog nutrition. Cereals such as corn, rice, sorghum, wheat, oats, barley, and others are mainly used as a source of carbohydrates in feed [5]. Plant components in food, especially the aforementioned cereals, can provide a breeding ground for the growth and development of microscopic filamentous fungi and their secondary metabolites, mycotoxins. The most common mycotoxins that can occur in cereals and other forages are deoxynivalenol and zearalenone [6].

Deoxynivalenol, also known as vomitoxin, is a type B trichothecene and epoxy-sesquiterpenoid produced primarily by the microscopic fungus *Fusarium graminearum*. It was first isolated from moldy barley grains and chemically described in 1970 [7]. It is soluble in water, ethanol, acetonitrile, and other polar solvents and is stable at high temperatures and low pH [8]. Deoxynivalenol is a natural toxicant that can negatively affect the health of animals. It has been reported that dogs with deoxynivalenol poisoning show similar clinical signs to pigs (vomiting, food refusal, and associated weight loss) [9]. Songsermsakul et al. (2007) describe, in addition to the negative impact of deoxynivalenol on the gastrointestinal tract of dogs, its immunosuppressive effects [10]. Consumption of feed contaminated with this trichothecene can lead to impaired immune resistance to pathogens in dogs, as well as to reactivation of chronic infections [11].

Zearalenone is one of the most important *Fusarium* mycotoxins. It is produced by several species, including *F. graminearum*, *F. culmorum*, *F. cereals,* and *F. equiseti*. This non-steroidal oestrogenic mycotoxin is mainly found in corn, wheat, oats, and barley and its production depends on cool temperatures and high humidity [12]. Zearalenone is a thermostable compound that does not degrade during the processing of feed materials and feed, even at high temperatures [13]. Female dogs are particularly sensitive to estrogens, and elevated concentrations of endogenous and/or exogenous hormones also may increase the risk of other systemic disorders [14]. After entering the body, zearalenone can cause acute vulvovaginitis, estrous cycle disorders, and impaired fertility [15].

According to EFSA (European Food Safety Authority) dogs are considered sensitive to zearalenone. Based on myometrium and endometrium lesions, enlargement and atrophy of uterine glands, blood haematology and biochemistry, the lowest observed adverse effect level (LOAEL) of 25 µg/kg body weight per day has been estimated for mature bitches [16]. As the presence of deoxynivalenol and zearalenone cannot be completely avoided, guidelines have been developed in the EU for maximum levels of mycotoxins in compound feed for various animals, including dogs (Table 1) [17].

The aim of this work was to determine deoxynivalenol and zearalenone in 14 samples of dry food for adult dogs using ELISA immunoassay.

MATERIALS AND METHODS

Samples

A total of 14 samples of dry dog food in the form of granules were examined. The samples were obtained from various commercial and specialized sellers and were intended for feeding young and adult dogs. The samples were divided into three categories: generic, popular, and

Table 1. Reference values of deoxynivalenol and zearalenone in products for animal animals feeding

Mycotoxins	Products intended for animal feeding	Guidance value in mg.kg^{-1} (ppm) relative to a feed with a moisture content of 12 %
Deoxynivalenol	Compound feed for:	
	pigs	0.9
	calves (< 4 months), lambs, kids and dogs	2
	other animals	5
Zearalenone	Compound feed for:	
	piglets, gilts, puppies, kittens, dogs and cats for reproduction	0.1
	adult dogs and cats other than for reproduction	0.2
	sows and fattening pigs	0.25
	calves, dairy cattle, sheep (including lambs) and goats (including kids)	0.5

Source: Commission Recommendation (2016/1319) [17]

premium. The generic feed group included samples of feed that did not bear the name of the manufacturer and were marketed locally or regionally. The second category, so-called popular feeds, included samples of feed sold in grocery store chains. The third category included premium feeds from specialized sellers.

Preparation of samples for deoxynivalenol determination

Representative samples (500 g each) were stored in a cool, dry, and dark place until analysis. Sample processing was carried out as follows: 100 ml of distilled water was added to 5 g of ground and homogenized sample. Then, a 3-minute shaking was performed, followed by filtration of the extracts through Whatman filter paper No. 1 (Cytiva, Kent, UK). 50 µl of the filtrates were used for the quantitative determination of deoxynivalenol according to the RIDASCREEN FAST DON protocol, quantitative test (R-Biopharm AG, Darmstadt, Germany).

ELISA analysis

The principle of the test is an antigen-antibody reaction. The wells in the microtiter strips are coated with capture antibodies directed against anti-aflatoxin antibodies. Standards (0 ppm; 0.222 ppm; 0.666 ppm, 2 ppm, 6 ppm), sample solutions, deoxynivalenol-enzyme conjugate, and anti-deoxynivalenol antibodies were added to the wells. Free and enzyme-conjugated aflatoxin compete for the binding sites of deoxynivalenol antibodies (competitive enzyme immunoassay). At the same time, antibodies against deoxynivalenol are bound by immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step using washing solution. Substrate/chromogen solution is added to the wells and incubated for 5 minutes in the dark. The bound enzyme conjugate converts the chromogen to a blue product. The addition of stopping solution results in a color change from blue to yellow. Measurement is performed photometrically at 450 nm, using an ELISA reader (Dynex Technologies, Inc., Chantilly, USA) where the absorbance is inversely proportional to the concentration of deoxynivalenol in the sample.

Preparation of samples for zearalenone determination

The analysis was performed using a commercial Veratox for zearalenone kit (Neogen Corporation, USA). The sample preparation procedure was as follows: 70 % methanol in amount of 25 ml was added to 5 g of ground sample. The samples were shaken for 3 minutes on an orbital shaker (Orbital Shaker – Biosan) and filtered through Whatman 1 filter paper. Filtrates were diluted with distilled water in a ratio of 1:5. 50 µl of diluted samples were used for the quantitative determination of zearalenone using an ELISA immunoassay.

ELISA analysis

The principle of ELISA analysis in the case of samples examined for the presence of zearalenone is the same as for the determination of deoxynivalenol. The standard solutions used in analysis contained zearalenone in the following concentrations: 0 ppb, 25 ppb, 75 ppb, 150 ppb, and 500 ppb. The resulting zearalenone concentrations (ppb; µg/kg) were read at the absorbance of 650 nm and were evaluated using an ELISA reader (Dynex Technologies, Inc., Virginia, USA).

RESULTS

Results of the quantitative determination of deoxynivalenol and zearalenone in samples of dry food for adult dogs are presented in Table 2. Concentrations of deoxynivalenol were below the detection limit of the test used (< 0.2 mg/kg; ppm) in all examined samples. Zearalenone was present in samples of generic and popular dry dog food samples below the detection limit of the test (< 5 ppb; µg/kg), and in 4 samples of premium dog food was zearalenone determined at concentrations of 21.799 ppb, 69.187 ppb, 96.745 ppb, and 163.463 ppb. In the remaining 6 samples of premium dog foods, the concentration was below the detection limit of the kit (< 5 ppb; µg/kg). It should be noted that none of the examined samples exceeded the recommended limits for deoxynivalenol (2 mg/kg; ppm) and zearalenone (0.2 mg/kg; ppm) in dog food as recommended by the Commission Recommendation (EU) 2016/1319 (Table 1).

DISCUSSION

A healthy and balanced diet using high-quality and safe ingredients is a modern phenomenon, not only in human nutrition but also in animal nutrition. Attention is also being paid to the nutrition of companion animals, which are

Table 2. Concentrations of deoxynivalenol (ppm; mg/kg) and zearalenone (ppb; µg/kg) in dry food for adult dogs

Type of feed for dogs	Samples (n=14)	Concentrations of deoxynivalenol	Samples (n=14)	Concentrations of zearalenone
generic	2	< 0,2 ppm	2	< 5 ppb
popular	2	< 0,2 ppm	2	< 5 pbb
premium	10	< 0,2 ppm	6	< 5 pbb
			4	> 5 ppb

sometimes considered family members. Dogs spend several years with us, so it is important to address the issue of the health safety of the food we offer them. Although dogs have no special requirements for carbohydrates, most feed manufacturers take advantage of dogs' ability to digest cereals and provide various types of cereals (corn, rice, wheat, barley, or sorghum, as well as cereal by-products) as a cheap source of energy. The proportion of these cereals in the feed formula can be up to 70 %, usually between 30 and 50 % [5]. The presence of cereals also brings with it the risk of various mycotoxins. Studies have shown that wheat contaminated with the mycotoxin deoxynivalenol is toxic even after 4 years of storage [18].

In our samples of dry dog food from various commercial and specialist suppliers, we did not detect deoxynivalenol in concentrations higher than the detection limit of the kit used (< 0.2 mg/kg; ppm). Concentrations of zearalenone were in 10 samples of dry dog food below the detection limit (< 5 ppb) and in 4 samples of premium dog food zearalenone was determined at concentrations ranging from 21.799 ppb to 163.463 ppb. Of the total number of 76 samples of dry dog food tested in Austria by ELISA, deoxynivalenol was present in 74 samples (97 %), and the maximum value of deoxynivalenol was > 250 µg/kg [19]. Seventy-six samples of dry dog food from 27 manufacturers were similarly purchased from retail stores, supermarkets, and specialist pet food stores. In addition to deoxynivalenol, Böhm et al. (2010) also detected the presence of zearalenone in the above-mentioned samples. Zearalenone was detected in 36 of the 76 samples examined (47 %) and maximum concentration of zearalenone was 298 µg/kg [19]. A similar study was conducted in Spain in 2020, where 60 samples of dry dog foods were examined (34 popular foods and 26 samples of premium foods), and 100 % of deoxynivalenol was detected in both cases. The authors also point out that in addition to deoxynivalenol, other mycotoxins such as aflatoxins, T2-toxin, ochratoxin A, fumonisins, and zearalenone were also found in the examined samples. Zearalenone was present in all premium foods (100 %) and in 88.2 % of supermarket foods in Spain in the above-mentioned samples. However, they point out that the examined feeds should not pose a risk of acute health complications in the form of mycotoxicoses for dogs [20]. In the past, studies have confirmed that dogs fed feed containing deoxynivalenol at 4.5 mg/kg of feed refuse food due to the reaction of the digestive tract to the mycotoxin [9, 10]. However, according to Songsermsakul et al., (2007), the aforementioned concentration of deoxynivalenol is not high enough to induce vomiting [10]. Zearalenone is toxic to both males and females [21]. According to a study by Boermans and Leung (2007), a dose of ~1 mg/kg zearalenone in pet food can cause infertility and negatively affect ovulation, implantation, pregnancy, and viability of the newborn [22]. In addition, daily intake of low doses of zearalenone could gradually damage the health of the dog [23] and may induce changes in metabolic processes in dogs that may cause hypoestrogenism [14, 24]. Given the presence of deoxynivalenol and zearalenone in our samples and in samples from foreign research, regular monitoring of mycotoxins in dry dog food would be necessary.

CONCLUSIONS

Mycotoxins can be a source of spoilage at any stage of feed production, processing, transport, storage, and consumption. Since there is relatively little information on the toxicological effects of mycotoxins on dogs, further studies are needed to determine the sources of mycotoxins that could contaminate feed intended for this type of companion animal. Future research primarily focuses on the issue of long-term administration of feed containing deoxynivalenol/zearalenone, particularly in relation to its immunosuppressive effects on the body and chronic diseases. In conclusion, it is necessary to state that the dog food samples examined in our study by us do not pose a risk of acute toxicosis in dogs, and it would be necessary to

determine the concentrations of other mycotoxins that may be the cause of impaired animal health.

Data Availability Statement

Data are contained within the article.

Conflict of Interest

The authors declare no conflicts of interest.

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Authors' Contributions

Conceptualization, M.H; methodology, M.H.; investigation, M.H., A.T.; data curation, M.H., A.H.Š.; writing—original draft preparation, M.H.; writing—review and editing, S.H., L.B.; funding acquisition, L.B.

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