

## **A survey of Deoxynivalenol and Zearalenone content in commercial dry foods for growing dogs**

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**Abstract:** *A survey of Deoxynivalenol and Zearalenone content in commercial dry foods for growing dogs.* The Polish market of commercial dry dog food for growing dogs of small breeds was surveyed for the presence of DON and ZEN with its metabolites  $\alpha$ - and  $\beta$ -zearalenol ( $\alpha$ -,  $\beta$ -ZOL). LC/MS method was applied for 6 randomly selected foods. The low levels of toxins found, gives overall picture of the safe segment of the market. The content of masked  $\alpha$ -zearalenol, however, from the perspective of long term consumption of small amounts may pose a health risk for the reproductive system of bitches.

*Key words:* mycotoxin, growing dog, reproduction

### **INTRODUCTION**

In recent years a growing concern can be observed, regarding the potential health risks for pets via grain compounds of petfood, commonly contaminated with mycotoxins (Böhm et al. 2010).

From the perspective of animal nutrition, the most significant mycotoxins or mycotoxin groups are aflatoxins, zearalenone – ZEN, deoxynivalenol – DON, fumonisins and ochratoxin A – OTA (Schatzmayer and Streit 2013).

Among the *Fusarium* mycotoxins, DON and ZEN are of special importance because their occurrence cannot be completely excluded during plant production. Special attention has to be paid to the co-occurrence of those toxins. The intensity

of the effects observed may depend both on the concentration and as well as on the DON/ZEN. The proliferation-depressing potential of DON and ZEN on the same target cells serves as an example of their metabolic interactions (Döll and Dänicke 2011).

Numerous studies confirm, that the severity of mycotoxicoses, occurring after ingestion of feed contaminated with mycotoxins depends on dose, exposure duration, type of toxin, animal species and the age of the animal (Zachiarasova et al. 2014).

The most apparent clinical effect of DON is the voluntary intake depression. Its acute ability to induce vomiting has assigned it the trivial name “vomitoxin” (Döll and Dänicke 2011). Hughes et al. (1999) revealed, that the toxicity of DON in dogs is similar to that in swine, while dogs can be more prone to vomiting, because of their well-known tendency to rapidly consume their food.

Administered orally, ZEN is entirely absorbed in the gastrointestinal tract. It can be metabolized in monogastric animals and humans with the formation of  $\alpha$ - and  $\beta$ -ZOL, subsequently conjugated with glucuronic acid. The anatomopathological investigations have revealed, that ZEN administration of 200  $\mu\text{g}/\text{kg}$  of body weight per os for 7 days, caused apparent

structural changes in the ovarian follicle of the young, growing bitch (Gajęcka et al. 2004). It should be stressed, however, that both male and female dogs are affected by ZEN toxicity (Boermans and Leung 2007).

The capacity for ZEN metabolism in plants, leading to  $\alpha$ - and  $\beta$ -ZOL synthesis was described for rice and corn by Goliński et al. (1988) and further confirmed in a model study (Berthiller et al. 2007). The presence of ZEN and its metabolites in animal feed triggers a competition with the body estrogens and leads to a deregulation of estrogenic effects via impaired RNA and protein synthesis, clinically resulting in hyperestrogenism and reproductive disorders (Döll and Dänicke 2011).

Numerous scientific reports have been published on the prevalence of various mycotoxins in foods for companion animals, in particular dogs but, to our best knowledge there was no previous reports addressing the mycotoxin contamination within particular segment of the market, i.e. dry food for growing dogs.

The aim of the present study was to survey the Polish market of commercial dry foods intended for puppies and growing dogs of small breeds, for the presence of DON, ZEN,  $\alpha$ - and  $\beta$ -ZOL.

## MATERIAL AND METHODS

### Sample collection

A product database was created containing 47 dry extruded foods for growing dogs of small and mini/toy breeds that are commercially available on the Polish market (including retail sale and internet distribution). Six foods were randomly selected and purchased in medium weight packages (from 0.4 to 3 kg), with the special care for the remote “best before” date. All products were stored factory-sealed until analysis.

Table 1 presents the nutritional characteristics of the evaluated products, as pictured on the labels, complemented with the calculated content of nitrogen free extract (NFE) and metabolizable energy (ME) levels.

TABLE 1. Analytical constituents listed on the labels with the calculated values of ME

Product number	Protein (%)	Fat (%)	Crude ash (%)	Crude fibre (%)	NFE (%)	ME (kJ/100g)
1	28.5	14.5	7.8	2.9	36.3	1 541.6
2	30.0	20.9	11.9	4.0	25.2	1 602.7
3	25.0	12.0	7.5	2.5	43.0	1 504.7
4	29.0	16.0	6.5	2.5	36.0	1 602.7
5	32.0	22.0	7.0	2.5	26.5	1 716.2
6	31.0	21.0	7.5	1.9	29.6	1 723.7

NFE – nitrogen free extract; ME – metabolizable energy (calculated accordingly to NRC 2006).

### LC/MS analysis

DON and ZEN were assessed in the samples according to norms PN-EN 15791 and PN-EN 15792, respectively.

In brief, the high performance liquid chromatographic method with fluorescence detection and immunoaffinity column clean-up was used.

Statistical calculations were performed using SPSS ver. 21 software.

## RESULTS AND DISCUSSION

The results of the mycotoxin content analysis were summarized in Table 2. All assayed substances were present in all products, however in small amounts. It can be noticed, that there was relatively little variation in ZEN and  $\beta$ -ZOL content, unlike with DON and  $\alpha$ -ZOL.

In the recently published paper, Błajet-Kosicka et al. (in press) collected data of 5 foods for puppies. Among other mycotoxins assayed, the average levels of DON and ZEN were substantially higher than those presented in this study.

The authors named the primary components of all 49 products studied: wheat, maize, soybean, rice, meat and animal-origin (chicken and fish) and dairy products (Błajet-Kosicka et al. 2014).

Main compounds of foods evaluated in this study were listed in Table 3. Products 1 and 2 were not produced with the extrusion technology. The label of the Product 2, probably intentionally did not contain the full list of ingredients, and there was no reply for the inquiry send by an e-mail to the producer.

Indeed, it is a general rule of thumb that the first 5 ingredients in the list provide 80% nutrients of the product (Case 2014). As it is helpful when comparing foods, it does not provide a definite knowledge on the possible source of toxins, since the potential contamination was shown not only for plant but also for animal commodities (Duca et al. 2009). In animals continuously fed on diets containing significant mycotoxin levels there is a risk of the varying extent of toxin deposition and accumulation beyond compliance even if the feed used

TABLE 2. The content of deoxynivalenol (DON) zearalenone (ZEN) and  $\alpha$ -,  $\beta$ -zearalenol ( $\alpha$ -,  $\beta$ -ZEL) in dry foods for growing dogs

Product number	DON ( $\mu\text{g}/\text{kg}$ )	ZEN ( $\mu\text{g}/\text{kg}$ )	$\alpha$ -ZEL ( $\mu\text{g}/\text{kg}$ )	$\beta$ -ZEL ( $\mu\text{g}/\text{kg}$ )
1	5.86	3.11	6.51	5.49
2	23.25	2.13	7.49	3.16
3	5.19	2.74	2.92	3.74
4	9.07	2.65	6.57	3.13
5	6.16	3.01	4.15	2.81
6	17.57	2.52	4.23	5.59
Average	11.18 $\pm$ 7.49	2.69 $\pm$ 0.35	5.31 $\pm$ 1.79	3.98 $\pm$ 1.24

TABLE 3. First 5 ingredients listed on the labels of assayed products

Product number	Ingredients				
	1	2	3	4	5
1	dried beef	brown rice	dried duck meat	maize	menhaden meat
2	beef	proprietary			
3	cereals (min. 4% maize)	meat and animal-origin products	plant protein extracts	oils and fats	plant derived products
4	maize flour	poultry meal	pork scratchings	poultry fat	bovine fat
5	poultry meat	rice	wheat meal	animal fats	wheat
6	dried chicken	rice	maize	chicken fat	dried fish

does not exceed the actual recommendation (Völkel et al. 2011).

Therefore, the final level of mycotoxins in the foods may be a cumulative effect of the contamination of various components, on the contrary to the previously published reports (Pestka 2007).

One plausible explanation of the noticeable differences between the results published by Błajet-Kosicka et al. (in press) and this study may be the quality of the ingredients used in particular production plants. It was previously claimed, that among the major challenges in the pet food industry are grain processing and sampling for the quality control (Leung et al. 2006).

Nevertheless, the content of  $\alpha$ -ZOL in all assessed foods may be slightly disturbing, thus demanding further studies of toxicological safety in pet foods. This metabolite is often described as more biologically dangerous than its parent chemical (ZEN). Early study (Fitzpatrick et al. 1989) has shown, that  $\alpha$ -ZOL has greater binding affinity for estrogen receptors, than ZEN or  $\beta$ -ZOL.

Consequently, we speculate that an applied, feasible for a dog owner outcome of the present study, could be the assumption of the theoretical risk, linked with the consuming of evaluated foods by a “model” animal. Table 4 presents the calculated dose of assayed toxins,

TABLE 4. Hypothetic approximate total exposure on mycotoxins ( $\mu$ g) of a model dog

Product number	DON	ZEN	$\alpha$ -ZOL	$\beta$ -ZOL
1	82.6	43.8	91.8	77.4
2 <sup>a</sup>	390.6–558.0	35.8–51.1	125.8–179.7	53.1–75.8
3	156.5	82.6	88.0	112.8
4	206.8	60.4	149.8	71.3
5	165.2	80.7	111.3	75.3
6	500.7	71.8	120.5	159.3

<sup>a</sup> Due to the given range of dosage, suggested by manufacturer.

that would be ingested by the dog during continuous offering of the particular food from 2 to 10 months of age (i.e. for 240 days – 8 months).

The model dog was set as a female of a small breed, with expected adult body weight of 5 kg, fed solely the particular product (1–6), consuming completely her daily ration, calculated accordingly to feeding suggestions, taken from the tables on the package. For the purpose of calculation, we assumed the constant level of mycotoxins in foods throughout the feeding period (batch to batch).

It seems reasonable to assume, that concentrations of DON and ZEN in dry pet foods, reported here, were below the no-observed-adverse-effect-level (NOAEL) proposed for dogs (EC 2002; Gajęcka et al. 2013b).

Boermans and Leung (2007) have described the main elements of the assessment of toxicity risk for pets, with its two main components being toxicity and exposure. Numerous reports emphasized chronic effects of consuming moderate to low amounts of mycotoxins as well as their co-occurrence or synergism (Leung et al. 2006, Döll and Dänicke 2011). Moreover, the special importance of fusariotoxins DON and ZEN interactions was discussed separately (Döll and Dänicke 2011).

Low doses of DON and ZEN (around NOAEL) administered in feed were shown to decrease the mRNA expression of genes controlling nitric oxide synthase (NOS), particularly in distal sections of the digestive tract of immature gilts (Gajęcka et al. 2013b). The prolonged effects of an impaired nitric oxide (NO) production can modify gastrointestinal functions, accelerating peri-

stalsis increasing tension of sphincters thus contributing to the inhibition of gastric emptying and digesta transfer in the intestines (Waśkiewicz et al. 2014).

Moreover, it should not be overlooked, that monotonic consumption of foods, containing small amounts (far below NOAEL) of mycotoxins, likely due to its cumulative effect in tissues, may result in female reproductive system dysfunctions, that were recently revealed (Gajęcka et al. 2013a).

Another physiologically important consequence of long term low dose oral exposure to ZEN was reported by Gajęcka et al. (2011). It was documented, that at 150% NOAEL dose of ZEN may disturb the fragile enzymatic equilibrium in pre-pubertal bitches by slowing down the steroidogenesis essential for instance in carbohydrate metabolism.

One interesting observation was increased body weight of pre-pubertal bitches intoxicated per os with NOAEL dose of ZEN: 50 µg/kg BW (Gajęcka et al. 2013a). Authors concluded, that very low doses of ZEN in commercial dog food do not result in clinical symptoms of intoxication, but they may enhance the somatic cells proliferation, stimulating/adaptive effect, consistent with the principle of hormesis (Calabrese 2005).

## CONCLUSIONS

The present study revealed low levels of DON, ZEN,  $\alpha$ - and  $\beta$ -ZOL in the dry food for growing dogs. The optimistic picture of obviously effective quality control in the manufacturing phase should not be overestimated, due to imminent adverse effects of the masked

mycotoxins of plant and animal origin, present in pet foods.

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**Streszczenie:** *Charakterystyka rynkowego segmentu suchych karm pełnoporcjowych dla psów pod względem poziomu deoksyniwalenolu i zearalenonu. W reprezentatywnej próbie 6 losowo wybranych produktów, należących do rynkowego segmentu suchych karm pełnoporcjowych dla psów rosnących, oznaczono poziom deoksyniwalenolu i zearalenonu wraz z jego metabolitami ( $\alpha$ ,  $\beta$ -zearalenolem), z wykorzystaniem metody LC/MS. Uzyskane wyniki wskazują na stosunkowo niski poziom oznaczanych mykotoksyn w ocenianych karmach. Istotne wydaje się zwrócenie uwagi na poziom  $\alpha$ -zearalenolu (zaliczanego do tzw. toksyn ukrytych, po ang. masked toxins), który w kontekście długotrwałego przyjmowania z karmą może stwarzać potencjalne zagrożenie dla rozwoju układu rozrodczego młodych suk.*

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