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Mycotoxin evaluation in feed for pets using tandem liquid chromatography mass/mass

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Abstract

Mycotoxins in food for pet nutrition have not been quite evaluated in Brazil. On the other hand, feed for animals to be used as raw material in the meat industry have been widely monitored. Pet feed industries, in parallel to the agri-business in the country have increased quite widely. Due to its demand, the quality of that type of feed became essential. Among the main mycotoxins responsible for feed contamination and that can cause serious harm to animal health are: (a) aflatoxins (AFLs: AFB_1, AFB_2, AFG_1 and AFG_2), (b) ochratoxin A (OTA), (c) zearalenon (ZON) and fumonisins (FBs: FB_1, FB_2, FB_3). Their toxicological effects in animals can cause damages to liver, kidneys, brain and/or gastrointestinal tract. Can also interfere to animal reproduction and be lethal. Considering the importance of feed safety for pets, a study was carried out aiming to evaluate the extent of mycotoxins contamination in feed for birds, cats, dogs, hamsters, horses, rabbits, pet-fish and turtle. A total of 123 samples of different animals and brands pet feeds were collected in pet-shops, veterinary clinics and supermarkets commercialized in Florianopolis city, Santa Catarina State, southern Brazil from February to July, 2006, also from horse stables. The total number of samples for each animal was: 19, 19, 46, 6, 26, 3, 3 and 1 for birds, cats, dogs, hamsters, horses, rabbits, pet-fish and turtle, respectively. The methodology used was liquid chromatography-mass/mass (LC-MS/MS) utilizing as ionization source *eletrospray* and *APCI* modes. All the samples presented some contamination of one or more than one mycotoxin surveyed, except for the turtle sample. As far as *type of animal feed* are concerned, the ones that presented contamination more often were for dogs (100 % - AFB_1), rabbits (100 % - AFB_1), hamsters (100 % - ZON), pet-fish (100 % - ZON), birds (94.7 % - AFB_1) and cats (84.2 % - AFB_1). Total AFLs levels varied from 0.879 to 209 µg kg^{-1}. The highest AFB_1 contamination was detected in hamsters´ feed with 185 µg kg^{-1} and the lowest in horse feed (1.14 µg kg^{-1}). Horse feed presented low levels of FB_1 ranging from 4.76 to 91.6 µg kg^{-1} (MRL: 5,000 µg kg^{-1}). Despite of the levels detected, only AFL total and ZON, corresponding to 6 (4.9 %) and 19 (15.5 %) of the total samples surveyed, presented levels above international regulations (50 and 50/100 µg kg^{-1} for AFLs and ZON, respectively).

Key words: pet feed, mycotoxins, LC- MS/MS, liquid chromatography.

Introduction

Most animal feed have some fungi or spores of fungi development, usually in low amounts. Fungi growth in feed is undesirable, as they can

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consume nutrients causing loss of energy, fat, protein and vitamins for the animal. That can result to low nutrition. Furthermore, fungi growth in feed makes it compacted, difficult to handle, lead to color alteration, different consistency and smell, thus being rejected by animals. Apart from that, they can produce mycotoxins. The main mycotoxins are associated to *Fusarium*, *Aspergillus*, *Penicillium* and *Claviceps* genera (Bruerton, 2001; Dawson et al., 2001). Mycotoxins cause economic losses for the domestic animals (pets) owners as they affect the animal health, reduce their productivity, increase the expenses with veterinaries and may lead to the animal death (Hussein and Brasel, 2001). The main mycotoxins responsible of feed contamination that may provoke serious animals´ health damages are: aflatoxins (AFLs: AFB_1, AFB_2, AFG_1, AFG_2), zearalenone (ZON), ochratoxin A (OTA) and fumonisins (FBs). Birds are more sensible to AFLs and OTA than horses and dogs and horses can be seriously affected by FBs (Fernandez et al., 1994). Younger/smaller the animals, more susceptible to these mycotoxins they are compared to older/bigger ones. Chronic AFLs toxicity can cause weight loss, immune suppression and liver alterations. Its acute toxicity can cause sub-cutaneous and intra-muscle hemorrhages, leading to death. Table 1 shows the fungi, mycotoxin, grain affected and the respective damage to different animals. The prevention of animal mycotoxicosis can be reached with the use of good quality cereals (low moisture content) as ingredients (Lazzari, 1997; Scussel, 2002). However it is important, more than the control of moisture and temperature during the storage of ingredients and feed, to monitor mycotoxin contamination through periodical analysis (Menegazzo, 2003, 2006). Feed quality should be monitored, not only for zoo-technical animals (bovines, swine, birds, fish), but also for pets (dogs, cats, rabbits, hamsters, horses). Therefore a survey was carried out on mycotoxin contamination in feed for pets analyzed by Tandem liquid chromatography mass/mass (LC-MS/MS) - a highly sensitive method - in the city of Florianopolis, Santa Catarina State (SC), southern Brazil.

Material and methods

Material

(a) Samples: 8 different types of pet feed (total of 123 samples) and brands (50) from pet shops of Florianopolis city, Santa Catarina State (SC), Southern Brazil. They were sold in packs of 500 g or 1 kg and for most of the feeds: in pellets. Except for horses: the packs were of 20

Table 1. Fungi and mycotoxin toxicological effects in feed for animal production.

Fungus	Mycotoxin	Affect grain	Damage on animals	Affected animal
Aspergillus	Aflatoxins	Maize Sorghum Cotton, peanut	Hepatotoxicity, imunosupression, intestinal hemorrhage, carcinogenicity	All species
Aspergillus *Penicillium*	Ochratoxin A	Maize Rice	Kidney degeneration	Swine and chicken
Fusarium	Zearalenone	Maize, rye Damaged grain	Reproduction effects	Swine and sheep
Fusarium	Fumonisins	Maize, damaged grains	Neurological effect	Equine, swine

Adapted from Bruerton (2001).

kg (mix of rye, wet oat and alfafa (*Medicago sativa* L.) and for pet-fish packs of 50 g.

(b) Standards: aflatoxins (AFLs: AFB_1, AFB_2, FG_1 e AFG_2), ochratoxin A (OTA), zearalenone (ZON) and fumonisin B_1 (FB_1). All from Sigma.

(c) Solvents: acetonitrile, methanol (HPLC grade), ammonium acetate (p.a.) and mili-Q water.

(d) Equipment: liquid chromatograph Model 1100, Agilent; mass/mass detector, Model API 4000, Applied Biosystems; mill, Series II, Romer and stainless steel blenders, Skymsen Eletro.

Methods

(a) Sample collection: samples were collected from pet shops, veterinary clinics, supermarkets and horse stables, as well as, donated from pet owners from February to July, 2006. The total number of samples for each animal was: 19, 19, 46, 6, 26, 3, 3 and 1 for birds, cats, dogs, hamsters, horses, rabbits, pet-fish and turtle, respectively. A portion of 150 g of each sample was transferred from original packs to polyethylene containers with toppers and stored in the freezer for analysis. Each sample was grinded in a mill and separated in 50 g portions for mycotoxin analysis in duplicate.

(b) Mycotoxin analysis: mycotoxins were extracted with acetonitrile and water (Spanjer et al., 2004) and quantified by LC-MS/MS (Xavier and Scussel 2006) using as ionization source *eletrospray* and *APCI*. The LOD and LOQ of the mycotoxins obtained with the method were as follows: *LOD:* 0.0125; 0.05; 0.05; 0.1; 0.04; 0.08 and 0.4 µg kg^{-1} for AFLs (AFB_1; AFB_2; AFG_1; AFG_2); ZON; OTA and FB_1, respectively. *LOQ:* 0.025; 0.1; 0.1; 0.2; 0.08; 0.16 and 0.8 µg kg^{-1}, respectively.

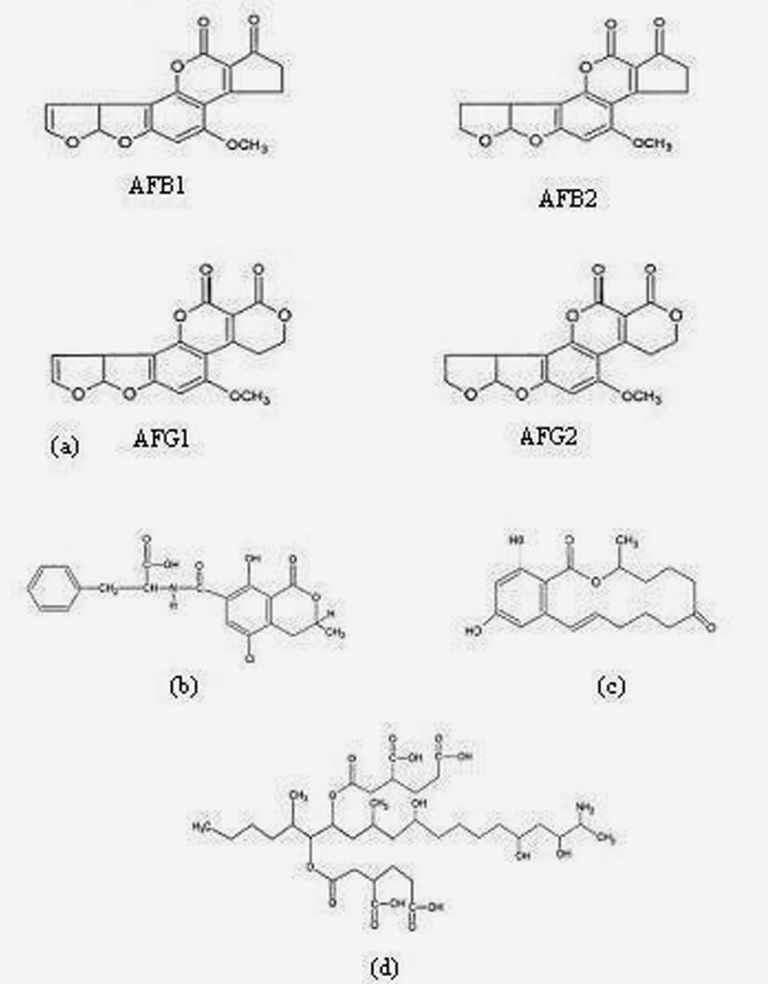

Figure 1. Chemical structures of (a) aflatoxins, (b) ochratoxin A, (c) zearalenone and (d) fumonisin B1.

Results and discussion

The percentage, per type of animal, of total pet food samples analyzed were: 38 % for dogs, 22 % for horses, 15 % for cats, 15 % for birds, 5 % for hamsters, 2 % for rabbits, 2 % for pet-fish and one percent for turtles (Figure 2). All the samples presented some contamination of one or more than one mycotoxin surveyed except for the turtle sample. Tables 2 and 3 show the number of positive samples and levels of contamination.

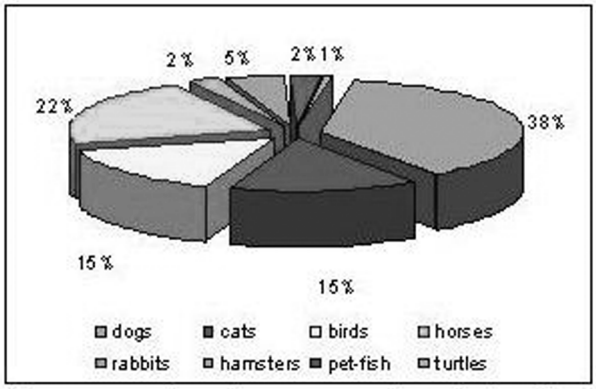

Figure 2. Percentage of samples *versus* species of animal feed surveyed.

Aflatoxin contamination: AFLs were detected in most of the samples analyzed and the levels ranged from traces (0.031) to 80.5 µg kg^{-1} for AFB_1. Only one sample of hamster feed showed a much higher level of AFB_1 (185 µg kg^{-1}). Indeed, hamster feed had peanuts in their composition, an ingredient that should have its quality (AFLs) checked before using it for feed production. All dogs and rabbits´ feed presented contamination with AFB_1. For total AFLs (AFB_1+AFB_2+AFG_1+AFG_2) the range was from 0.879 to 209 µg kg^{-1}. Out of all 123 samples analyzed, 81.30 % (100 samples) were contaminated with AFB_1, 52.8 % (65) with AFB_2, 81.3 % (100) with AFG_1 and 85.4 % (105) with AFG_2. The minimum and maximum levels for each toxin and for total AFLs are shown in Table 3. As far as AFL total is concerned, in samples of rabbit feed the range was between 2,594 and 172.30 µg kg^{-1} of total AFLs. Considering the number of feed samples contaminated above the MRL (50 µg kg^{-1}) reported by FAO (FAO, 2004) for total AFLs (AFB_1+AFB_2+AFG_1+AFG_2), it was found only six samples (4.9 % of the total) contaminated. Those rejected samples were: two (66.7 %) of rabbits, one (2.17 %) of dogs, two (10.5 %) of birds and one (16.7 %) of hamsters feed.

Table 2. Number of positive pet feed samples according to type of mycotoxin and animal Species Animal.

Species	Number of sample	Number of positive samples (%)						
		AFLs[a]				ZON[b]	OTA[c]	FB_1[d]
		AFB_1	AFB_2	AFG_1	AFG_2			
Horse	26	16(61.5)	ND	21(80.7)	21(80.7)	9(34.6)	4(15.4)	8(30.7)
Cat	19	16(84.2)	10(52.6)	15(78.9)	18(94.7)	10(52.6)	6(31.6)	NA[f]
Bird	19	18(94.7)	15(78.9)	17(89.4)	19(100)	8(42.1)	3(15.8)	NA
Dog	46	46(100)	33(71.7)	43(93.4)	44(95.6)	31(67.4)	26(56.5)	NA
Rabbit	3	3(100)	3(100)	2(66.7)	2(66.7)	2(66.7)	2(66.7)	NA
Hamster	6	1(16.6)	4(66.7)	2(33.3)	1(16.6)	6(100)	ND	3(50)
Pet fish	3	ND[e]	ND	ND	ND	3(100)	ND	NA
Turtle	1	ND	ND	ND	ND	ND	ND	NA
Total:	123	100(81.3)	65(52.8)	100(81.3)	105(85.4)	69(56.1)	41(33.3)	11(8.9)

[a] aflatoxins [b] zeralenone [c] ochratoxin A [d] fumonisin B_1

[e] not detect [f] not analyzed

Table 3. Levels of mycotoxins detected by LC/MS/MS in pet food from Southern Brazil (February to July, 2006).

Pet food	Mycotoxins (µg kg^{-1})									
	AFLs[a]									
	AFB_1		AFB_2		AFG_1		AFG_2		Total AFLs	
	min.	max.	min.	max.	min.	max.	min.	max.	min.	max.
Cat	0.114	4.76	0.135	4.38	0.868	2.95	3.04	15.5	3.04	21.4
Bird	0.224	4.77	0.357	9.04	1.23	4.67	2.87	153	3.09	167.5
Dog	0.453	7.67	0.569	7.05	0.737	6.24	2.94	56.1	2.18	66.4
Rabbit	2.13	80.5	0.464	15.2	9.45	17.3	23.4	59.3	2.59	172.3
Hamster	ND[e]	185	0.482	24	3.75	5.95	ND	0.995	0.879	209
Pet fish	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Turtle	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Horse	Tr[f](0.031)	1.14	0.108	1.68	1.17	4.29	2.14	11.8	1.75	14.7

Pet food	Mycotoxins (µg kg^{-1})					
	ZON[b]		OTA[c]		FB_1[d]	
	min.	max	min.	max.	min.	max.
Cat	0.86	51.4	10.6	85.8	NA	NA
Bird	3.11	750	6.33	19.7	NA	NA
Dog	2.14	313	2.44	97.5	NA	NA
Rabbit	61.2	86.6	34.1	43.1	NA	NA
Hamster	10.7	133	ND	ND	3.79	10.4
Pet fish	0.834	4.48	ND	ND	ND	ND
Turtle	ND	ND	ND	ND	ND	ND
Horse	0.135	7.03	0.383	20.1	4.76	91.6

[a] aflatoxins [b] zeralenone [c] ochratoxin A [d] fumonisin B_1 [e] not detect [f] not analyzed [f] traces

LOD: 0.0125; 0.05; 0.05; 0.1; 0.04; 0.08 and 0.4 µg kg^{-1} for AFLs (AFB_1; AFB_2; AFG_1; AFG_2); ZON; OTA and FB_1, respectively.

LOQ: 0.025; 0.1; 0.1; 0.2; 0.08; 0.16 and 0.8 µg kg^{-1} for AFLs (AFB_1; AFB_2; AFG_1; AFG_2); ZON; OTA and FB_1, respectively.

Zearalenone and Ochratoxin A: from the total of feed samples, 56.1 and 33.3 % were contaminated with ZON and OTA. The values for ZON had varied between 0.135 - 750 µg kg^{-1}, OTA was present in levels ranging 0.385 - 97.5 µg kg^{-1}. International regulation for those toxins can vary depending on the feed and country. For ZON the MRL is from 50 - 100 µg kg^{-1} and for OTA is from 100 to 200 µg kg^{-1}. They depend on the animal species. Despite of that, from the pet feed surveyed (123) for OTA and ZON, only 19 samples had higher levels than the ZON MRL. The highest incidence of contamination occurred in the feed for dogs (313 µg kg^{-1}). It was found ZON also in cats, rabbits, birds and hamsters (51.4; 750; 86.6 and 133 µg kg^{-1}) pet, thus *above* the international MRLs. On the other hand, the *lowest* values for both toxins were found in horse feed: 0.135 to 7.03 and 0.383 to 20.1 µg kg^{-1}, respectively. OTA was not detected in feeds for hamsters, pet-fish and turtle (under the method LOQ).

Fumonisins: eight horse samples and three hamsters´ were positive for FB_1. The average levels were 25.35 and 6.72 µg kg^{-1} (min. 4.76 and 91.6; max 3.79 and 10.4 µg kg^{-1}), respectively. FDA

recommends an MRL of FBs ($FB_1 + FB_2 + FB_3$) of 5 mg kg^{-1} (FDA, 2001, 2005; Günter, 2003), therefore the samples had international acceptable levels.

It is important to emphasize, that all feeds samples had maize in their composition, except for hamsters and turtle. That ingredient may be directly related to the contamination of the samples for toxins surveyed.

Conclusions

Considering the safety of the feed surveyed in this work, it is possible to conclude that the animals (hamsters, birds, rabbits, cats, dogs and horses) would be more in harm. If they would eat those feed analyzed which were contaminated with higher levels than the MRL (AFLs, OTA and ZON) probably would develop symptoms related to liver, kidneys, fertility hormones and gastric intestinal tract. Depending of the level of contamination it could lead to animal death. It is important to note that small animals (hamsters, birds, rabbits, small dogs/cats) are more susceptible to those mycotoxins than bigger ones (horses and dogs).

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