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Iffat Tahira
Romer Labs Pakistan, Rawalpindi

Nighat Sultana
Institute of Natural and Management Sciences, Rawalpindi

Nafeesa Qudsia Hanif
Fatima Jinnah Women University, Rawalpindi, nafeesahanif9@gmail.com

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IDENTIFICATION OF AFLATOXINS AND OCHRATOXIN A IN SELECTED IMPORTED PET FOOD

Iffat Tahira[1], Nighat Sultana[1,3] and Nafeesa Qudsia Hanif[1,2,*]

[1]Romer Labs Pakistan, Rawalpindi, Pakistan.

[2]Institute of Natural and Management Sciences (INAM), Rawalpindi, Pakistan.

[3]Fatima Jinnah Women University, Rawalpindi, Pakistan.

[*]Email: nafeesahanif9@gmail.com

ABSTRACT

The current study was conducted to analyze the aflatoxins (AF i.e. AFB_1, AFB_2, AFG_1 AFG_2) and ochratoxin A (OTA) in imported pet food. A total of five hundred and ten commercially available imported pet food samples of cat (solid = 240, semi-solid = 90) and dog (solid = 150, semi-solid = 30) were collected from retailers and analyzed by chromatographic technique i.e. HPTLC. Results revealed 45.83% (mean, 3.90 ppb) and 18% (mean, 4.83 ppb) of AFB_1 incidence in solid pet foods of cats and dogs, respectively. However, lower levels i.e. 8.88% (mean, 4.60ppb) and 6.66% (mean, 2.80ppb) of AFB_1 were observed for semi-solid food samples of cat and dog, respectively. Aflatoxin B_2 was found in solid cat food only with an incidence of 12.5% (mean, 0.89 ppb). About 35.33% (mean, 4.5 ppb) and 26.66% (mean, 2 ppb) of OTA were observed in solid and semi-solid dog foods respectively. Similarly, the trend of OTA in cat foods was 15% (mean, 3.87 ppb) and 13.13% (mean, 1.0 ppb) for solid and semi-solid foods. Furthermore, co-contamination of AF and OTA were observed in 33.33% and 41.60% samples of dog and cat foods, respectively. It was concluded that natural incidence of mean contaminations was below than European Commission (EC) legislation i.e. 20 ppb and 10 ppb for AF and OTA, respectively. However, co-occurrence of mycotoxins in pet food may exert synergistic deleterious effects even at levels far below the regulatory limits.

Keywords: Pet food, Aflatoxins, Ochratoxin A, EC regulations.

INTRODUCTION

Pet foods are basically balanced diets, including calories of protein, fat, carbohydrates, vitamins and minerals required to sustain life and optimize performance (Zicker, 2008). Pet food is mainly prepared by using different ingredients i.e. cereals (corn, wheat gluten, corn gluten and rice protein) and meat meal (Leung *et al.,* 2006). These raw materials, particularly cereals, are considered to be an ideal substrate for the fungal growth that might lead to production of mycotoxins. It is reported that pet food may contain mycotoxins higher concentrations than raw cereals due to processing (Brera *et al.*, 2006).

Mycotoxins are toxic secondary metabolite products of molds generally produced by *Fusarium*, *Aspergillus* and *Penicillium* species and found on common feed stuff, particularly cereals. They may cause different toxic effects in animals called "mycotoxicosis", varying from immune suppression, estrogenic or neurotoxin effects, and may lead to death in severe cases (Leung *et al*., 2006). Several mycotoxins outbreaks have been reported in

the past few years (Fadeyemi and Akinrinde, 2012; Maia *et al*., 2002). According to recently published mycotoxins survey, raw materials that are being used in commercial pet foods are frequently contaminated with mycotoxins (Khatoon *et al*., 2012; Striet *et al*., 2012). Contamination of pet food with mycotoxins poses a serious health threat to pets, causing an emotional and economical concern to pet owners. Among these, aflatoxins (AF) and ochratoxin A (OTA) represent major toxic threats in animal feed due to their carcinogenic and nephrotoxic effects (CAST, 2003). The subject of mycotoxin contamination incidence is well reviewed in small animals. However, scientific literature on mycotoxicosis in pets is rare (Puschner, 2002). The mycotoxins contamination in pet food is an unveiled area in Pakistan. The present study was planned to assess the prevalence of mycotoxins in selected imported pet foods.

MATERIALS AND METHODS

Sample Collection

A total of five hundred and ten (n = 510) imported pet food samples, including cat food (solid, n = 240; semi-solid, n = 90) and dog food, (solid, n = 150; semi-solid, n = 30) were randomly collected from local retailers. The origin of pet food samples were the EU, the UK and Korea. Samples were then stored at 4°C for future mycotoxin analysis.

Sample Preparation and Toxins Determination

A method described by Sultana *et al.*, (2013) was adopted for the quantification of total aflatoxins and ochratoxin A. For this, twenty five grams of well-ground (20 mesh) pet food samples were accurately weighed and blended for 3 minutes at high speed (Osterizer, Germany) with 100 mL of (methanol:water;80:20; v/v) and filtered by Whattman No. 1. After filtration, about 8 mL of phosphate buffer saline (PBS) was added in 4 mL of sample extract. This was re-filtered to remove the fat layer if a semisolid food sample was present. The pH was adjusted to 7.0 and 7.4 by using 0.1M NaOH or HCl as required for AF and OTA, respectively. After, the pH adjustment sample extract was loaded on the AflaStar^{TM}IAC and OchraStar^{TM}IAC (Romer, Austria) at the rate of 1 mL/minute, respectively. Upon completion of loading, columns were washed with 20 mL distilled water at a flow rate of 3 mL/minute. Then aflatoxins and ochratoxin A was eluted with 3 mL of methanol at 0.5 mL/minute. The eluent was dried at 60 ºC and re-dissolved in 400 µL of (toluene: acetonitrile; 95:5: v/v) and (toluene: acetic Acid; 99:1; v/v) for AFs and OTA, respectively. A volume of 40 and 100 µL of reconstituted samples were spotted on separate HPTLC silica gel 60 plates along with different concentrations of AF and OTA standards (Biopure, Austria). Limit of detections of adopted method were 0.1 ppb for AFB_1, AFG_1, OTA and 0.50 ppb for AFB_2, AFG_2.

RESULTS

A total of five hundred and ten (n = 510) pet food samples were analyzed for the aflatoxins and ochratoxin A contamination. Out of these, three hundred and thirty samples were of cat food (i.e. solid = 240, semi-solid = 90) and one hundred and eighty (i.e. solid = 150 and semi-solid = 30) were of dog food. Among all total analyzed samples, 28.82% (n = 147) samples were found positive for AFB_1, 5.80% (n = 30) of AFB_2 and 21.37% (n = 109) for OTA. However, neither cat food nor dog food was found contaminated for AFG_1 and AFG_2 (Figure1).

Interestingly, findings of the present

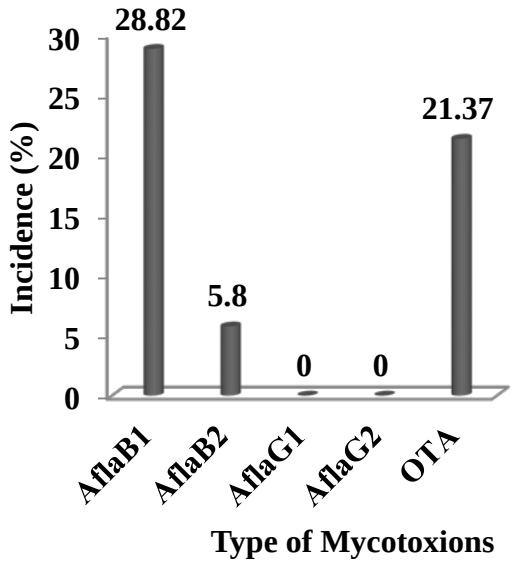

Figure 1: Incidence (%) of aflatoxins and ochratoxin A in solid and semi-solid cat and dog food samples

study revealed the higher incidence of AFB_1in solid types of pet foods i.e. 45.83% (range, 0.5-9 ppb; mean, 3.90±0.89ppb) and 18% (range, 0.5-8 ppb; mean 4.83±1.01 ppb) of cat and dog foods, respectively. However, for the semi-solid type, 8.88% (range, 0.2-7.5 ppb; mean, 4.60 ± 1.25 ppb) and 6.66% (range, 0.5-9 ppb; mean, 2.80±1.45 ppb) were recorded for cats and dogs pet foods, respectively. Aflatoxin B_2 was only found in solid cat food with an incidence of 12.5%, ranging from 0.1 to 3.75 ppb (mean 0.89 ± 0.97 ppb). As far as OTA contamination is concerned, dog foods were tainted with relatively higher levels i.e. 35.33% (range, 0.1-20 ppb; mean, 4.5±0.93 ppb) and 26.66% (range, 0.1-8 ppb, mean 2.0±1.52 ppb) for solid and semi-solid types, respectively. Similarly, the presence of OTA in cat foods was 15% (range 0.1-18 ppb; mean, 3.87±1.05 ppb) and 13.13% (range, 0.1-4ppb; mean 1.0±.37 ppb) for solid and semi-solid foods as shown in Table 1. The results of the present study showed 8.33, 25 and 8.33 percent of cat food samples were simultaneously contaminated with AFB_1, AFB_2; AFB_1, OTA &AFB_1, AFB_2andOTA combinations, respectively. While, 33.33 percent of dog food samples were co-contaminated with AFB_1 and OTA only (Figure 2).

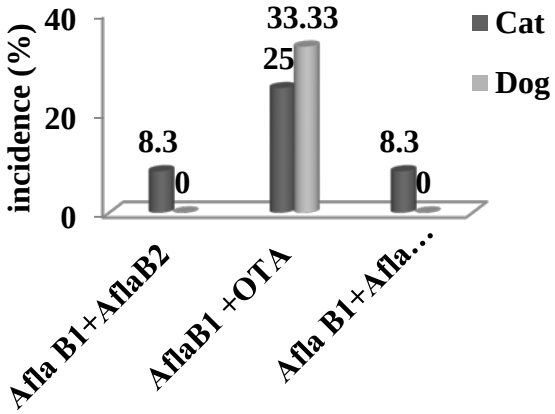

Figure 2: Co-incidence of aflatoxins and ochratoxin A in solid and semi-solid pet food samples.

DISCUSSION

Results of present findings revealed a higher percentage incidence of mycotoxin in solid pet food. Ubiquitously distributed *Aspergillus flavus* might be responsible for elevated incidence of aflatoxins in solid pet food samples. Solid pet food is basically constituted of cereals i.e. peanuts, sorghum, soya, rice and corn. According to the study of Khatoon *et al.*,(2012), corn is considered as an ideal substrate for the aflatoxins producing fungi. Similarly Zaki *et al*. (2012) pointed out that mold growths on grains under field conditions or during storage occur at moisture levels above 16% and at temperatures above freezing 10-40ºC. Germination of *Aspergillus flavus* spores leads to aflatoxin production. Aflatoxins are well documented for their toxic effects and have been classified as a class 1 carcinogen

(IARC, 2002). Among pets, and especially for

Table 1: Scenario of mycotoxins in solid and semi-solid pet food samples.

Pets	Food type	N	Aflatoxin B_1 (ppb)		Aflatoxin B_2(ppb)		Ochratoxin A (ppb)	
			Positive (%)	Mean±SD (min-max)	Positive (%)	Mean±SD (min-max)	Positive (%)	Mean±SD (min-max)
Cat	Solid	240	110 (45.83)	3.90±0.89 (0.5-9.0)	30 (12.5)	0.89±0.97 (0.1-3.75)	36 (15)	3.87±1.05 (0.1-18)
	Semi Solid	90	8.0 (8.88)	4.60 ±1.25 (0.2-7.5)	ND		12 (13.13)	1.0 ± 0.37 (0.1-4)
Dog	Solid	150	27 (18)	4.83±1.01 (0.5-8.0)	ND		53 (35.33)	4.5± 0.93 (0.1-20)
	Semi-solid	30	2 (6.66)	2.80±1.45 (0.5-9.0)	ND		8.0 (26.66)	2.0±1.52 (0.1-8)

ND – Not detected

cats and dogs, it is associated with jaundice, lack of energy, vomiting, and in severe cases death (Hussain *et al*., 2001), due to ingredients that have been defined by various countries around the world. In Pakistan, regulatory limits have only been defined for poultry feed i.e. 20ppb. However, present findings showed that imported pet food is safe for consumption, as observed mean level of aflatoxin B_1 in all four types of food were recorded below the regulatory limit i.e. 20ppb (European Commission, 2007). It may be due to the reason that all collected samples were imported from the countries UK, Korea, and the USA. These countries strictly follow the mycotoxins regulation in raw materials according to their region. Several studies reported that commercial dog and cat foods were likely to contain lower aflatoxins contamination when compared with those of birds, cattle and other species (Sharma and Marquez, 2001; Maia *et al*., 2002). Although, the percentage of positive samples varies by surveys, almost all the positive samples contained less than 20ppb AFB_1. The results obtained from the present study also seem to confirm the trend observed in the previous surveys Puschner, 2002; Leung *et al*., 2006).

Ochratoxin A is a potent renal mycotoxin that widely contaminates agriculture commodities and, being a nephrotoxic, is also classified as a class 2B possible carcinogen by the IARC. For animal feed, the European Commission has also defined 10ppb as regulatory limit (EC, 2007). Observed mean levels were within agreeable limits. However, few samples were noticed with slightly higher levels than EC regulations i.e. 16 and 20ppb for cats and dog food, respectively. These high levels can't be ignored as literature suggested that pets, i.e. cats and dogs, are more susceptible than small ruminants and dairy animals for OTA toxicity (Zaki *et al.*, 2012). General symptoms associated with OTA are poisoning, weight loss, vomiting, dehydration, bloody diarrhea, temperature and anorexia (Boermans and Leung, 2007).

Besides, pet food co-contamination is a matter of great concern. According to Striet *et al* (2012) mycotoxins in combination may exert synergetic, additive or antagonistic effects. According to a previous study, co-occurrence of AF and OTA exerts their toxic effects synergistically. These effects are more lethal even at low level of individual mycotoxins (Boermans and Leung, 2007).

In conclusion, the present study indicated the existence of mycotoxins contamination in imported pet food. However, average levels were within acceptable limits, therefore, imported pet food samples are safe to consume. It is first reported that the study regarding mycotoxin contamination in imported pet foods in Pakistan provides the basis for further studies to assess other mycotoxins like metabolites of aflatoxins like aflatoxicol, aflatoxin M_1, zearalenone and trichotheces etc. Aflatoxins metabolites like aflatoxicol should be screened, as pet food contains not only cereals but meat, egg, fish portions also. Therefore, regular screening of mycotoxins in pet food is necessary to provide mycotoxins free food to pets.

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