Identification of Aflatoxins and Ochratoxin A in Selected Imported Pet Food

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The current study was conducted to analyze the aflatoxins (AF i.e. AFB1, AFB2, AFG1 AFG2) 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 AFB1 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 AFB1 were observed for semi-solid food samples of cat and dog, respectively. Aflatoxin B2 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.
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 Whatman 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™iac and OchraStar™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 AFB1, AFG1, OTA and 0.50 ppb for AFB2, AFG2.

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 AFB1, 5.80% (n = 30) of AFB2 and 21.37% (n = 109) for OTA. However, neither cat food nor dog food was found contaminated for AFG1 and AFG2 (Figure1).

Interestingly, findings of the present
Figure 1: Incidence (%) of aflatoxins and ochratoxin A in solid and semi-solid cat and dog food samples.

The study revealed the higher incidence of AFB1 in solid types of pet foods i.e. 45.83% (range, 0.5-9 ppb; mean, 3.90±0.89 ppb) 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 B2 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, 0.1-4 ppb; 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 AFB1, AFB2, AFB1, OTA & AFB1, AFB2 and OTA combinations, respectively. While, 33.33 percent of dog food samples were co-contaminated with AFB1 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 10-40°C. 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 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).

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

<table>
<thead>
<tr>
<th>Pets</th>
<th>Food type</th>
<th>N</th>
<th>Aflatoxin B$_1$ (ppb)</th>
<th>Aflatoxin B$_2$ (ppb)</th>
<th>Ochratoxin A (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive (%)</td>
<td>Mean±SD (min-max)</td>
<td>Positive (%)</td>
</tr>
<tr>
<td>Cat</td>
<td>Solid</td>
<td>240</td>
<td>110 (45.83)</td>
<td>3.90±0.89 (0.5-9.0)</td>
<td>30 (12.5)</td>
</tr>
<tr>
<td></td>
<td>Semi-Solid</td>
<td>90</td>
<td>8.0 (8.88)</td>
<td>4.60±1.25 (0.2-7.5)</td>
<td>ND</td>
</tr>
<tr>
<td>Dog</td>
<td>Solid</td>
<td>150</td>
<td>27 (18)</td>
<td>4.83±1.01 (0.5-8.0)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Semi-solid</td>
<td>30</td>
<td>2 (6.66)</td>
<td>2.80±1.45 (0.5-9.0)</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND – Not detected
Besides, pet food co-contamination is a matter of great concern. According to Striet et al. (2012) mycotoxins in combination may exert synergistic, 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 M₁, 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.

REFERENCES


Tahira et al.; Aflatoxins and Ochratoxin A in Imported Pet Food


