Full Length Research Paper

Aflatoxin status of some commercial dry dog foods in Ibadan, Nigeria

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The occurrence of aflatoxins B1, B2, G1 and G2 in commercial dry dog foods in the city of Ibadan, Southwest Nigeria, was investigated. Dry dog food samples from 6 producers were purchased on five different occasions from retail outlets in Ibadan, Nigeria. High performance liquid chromatography was used for separation and quantification of aflatoxin fractions, after consideration of the limits of detection and quantification of the method. Results indicate that aflatoxins B1, B2, G1 and G2 were detected in all the samples investigated, with B1 being the most abundant. The range of concentration of total aflatoxins was 7.76 to 11.93 µg/kg (mean: 9.61 µg/kg). The results show that dry dog foods marketed in Ibadan are frequently contaminated with aflatoxins, exposing dogs to adverse effects of aflatoxicosis. Scientifically based regulations for the acceptable limit of mycotoxins in pet foods in the country would be beneficial.

Key words: Aflatoxins, dog, contamination, Nigeria, toxicity.

INTRODUCTION

Contamination of pet food with mycotoxins poses a serious health threat to pets, causing an emotional and economical concern to the pet owners. Aflatoxins, among other mycotoxins, represent a major toxic threat in animal feeds. Aflatoxins are highly toxic and carcinogenic metabolites of fungi of the genus Aspergillus. The major fungi that produce these mycotoxins are Aspergillus flavus and Aspergillus parasiticus. Aflatoxins are commonly found in corn, peanuts, cottonseed, milk and tree nuts which are commonly used as dog food ingredients (Haschek et al 2002). Aflatoxins B1, B2, G1 and G2 are four naturally-occurring forms of aflatoxins, with aflatoxin B1 being the most potent, prevalent and carcinogenic (Puschnier, 2002; IARC, 1993). Aflatoxin production results when there are specific environmental temperatures and moisture conditions, and risk of contamination is increased when crops are stressed by drought, insect damage, improper field management or inappropriate handling or storage.

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There have been several reports on mycotoxin surveys of commercial pet foods, including those done in the United Kingdom (Scudamore et al., 1997), Brazil (Maia et al., 2002; Scussel et al., 2006) and United States (Henke et al., 2001). Similarly, a number of reported mycotoxin outbreaks in pet species resulting from consumption of contaminated commercial foods have also been documented in south eastern United States (Bailey and Groth, 1959), Pretoria, South Africa (Bastianello et al., 1987) and Korea (Jeong et al., 2006). Aflatoxins have been the most common cause of acute mycotoxin outbreaks in commercial dog food and corn is the usual source of aflatoxins in these cases. These available reports may, however, be insufficient to provide the whole picture of the mycotoxin problem associated with pet foods since only a small number of food poisoning cases are published. Again, many cases of mycotoxin poisoning are usually misdiagnosed. According to Boermans and Leung (2007), veterinarians often overlooked mycotoxins as the cause of chronic diseases such as liver and kidney fibrosis, infections resulting from immune-suppression and cancer.

The clinical syndrome of canine aflatoxicosis manifests
either as acute, sub-acute or chronic cases. Signs of acute intoxication may include lethargy, anorexia, icterus, gingival petechial hemorrhages, severe depression, polydipsia, polyuria, vomiting and sudden death in some cases. Chronic aflatoxicosis is caused by consumption of diets containing small to moderate amounts of aflatoxins continuously or intermittently. Dogs or cats will show clinical signs similar to sub-acute aflatoxicosis with a prominence of jaundice. Cancer is another long-term effect of aflatoxins. Lesions including hepatomegaly and a diffuse yellow friable appearance of the liver have been consistently identified in some reports of dogs diagnosed with sub-acute aflatoxicosis (Stenske et al., 2006).

Unpublished reports of some cases presented at the Veterinary Teaching Hospital, University of Ibadan, involving dogs indicate clinical signs and post-mortem findings that suggest the possibility of chronic poisoning most probably due to consumption of commercial compounded dog foods contaminated with mycotoxins. There is a dearth of information on the mycotoxin status of animal feedstuffs in Nigeria. The only report on incidence and level of contamination of animal feedstuffs in Nigeria was that of Shetty et al. (1987). The investigation, however, did not include pet foods. This work was therefore designed to provide information on the aflatoxin status of dry commercial pet foods in Nigeria. It is hoped that the study would serve as a prelude to determine guidelines and legislated limits for aflatoxins and other common mycotoxins in commercial dog feed in Nigeria.

MATERIALS AND METHODS

Six brands of dry dog foods were purchased in pet shops in the City of Ibadan, Nigeria from October, 2010 to March, 2011. These brands represented the majority of dog food marketed in the city, usually packaged as 2 kg bags. Purchases were made on five different occasions in order to minimize the possibility of obtaining a non-representative lot of food.

Preparation of samples

For each batch of purchases made, aliquots (about 50 g) from each 2 kg bag were pooled in separate sample bottles for aflatoxin analysis. They were identified by code letters CCF, DTF, MF, PF, SP and TF to preserve the privacy of the manufacturers. All samples were delivered to the laboratory at ambient temperature and stored at +4°C until initial sample preparation, after which they were stored at -20°C until required for analysis. Samples were allowed to defrost to ambient temperature prior to analysis. Aflatoxin analysis was performed using high performance liquid chromatography (HPLC) on each batch of the different brands of dog food according to the method of Rodricks and Stoloff (1970).

Extraction of aflatoxin from samples

Briefly, 50 g of food samples were carefully pipetted into a 250 ml conical flask and 5 g NaCl were added. One hundred millilitre (100 ml) of an 80:20 (%v/v) methanol-water mixture was then added and the mixture was homogenized for 5 min. The mixture was then filtered through a Whatman No 1 filter paper and 5 ml of the filtrate were transferred to a glass beaker to which 20 ml of a 90:10 (%v/v) solution of Triton 20 water was added. The solution was then filtered through Whatman No 5 filter paper. Four milliter (4 ml) of the filtrate were transferred to a glass reservoir with a Vican with a flatte immun-affinity column (Vican, Watertown, MA, USA) attached. Filtrate containing aflatoxins was passed through this gel suspension. The bound aflatoxins in the column were released from the antibodies following subsequent elution with methanol.

Separation of aflatoxin fractions

Analysis of aflatoxins in the samples was performed using high performance liquid chromatography. A HPLC system, Waters 616/626 model (Waters, Ireland) was used. Prior to aflatoxin analysis, the HPLC system was constructed using aflatoxin calibration standards with concentrations of 0.00, 1.56, 3.12 and 6.24 µg/l for B1; 0.00, 0.51, 1.01 and 2.02 µg/l for B2; 0.00, 0.98, 1.96 and 3.92 µg/l for G1 and 0.00, 0.55, 1.10 and 2.20 µg/l for G2.

The HPLC system was interfaced with a computer system and a software having a standard equation generated from the relationship of the intensity of the analyte in the samples against the concentration of aflatoxin standards. The analyte of the unknown was calculated by the software using the standard equation. The chromatographic separation was performed on a Hichrom-Hypersil H5ODS C18 column (4.4 x 250 mm id; Hichrome Ltd, Reading, UK). The column was maintained at ambient temperature with a flow rate of 1.0 ml/min. Aflatoxin was detected at the excitation and emission wavelengths of 365 and 450 nm, respectively. Aflatoxin concentration for the respective fractions was calculated thus:

\[
\text{Aflatoxin (µg/kg)} = \frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard} \times \text{weight of sample}}
\]

Statistical analysis

All data were calculated by Microsoft Excel. Results were reported as mean values and standard deviation (in µg/kg) for five determinations of the concentration of aflatoxin fractions from each brand of dog food sampled.

RESULTS

The detection limits for aflatoxin were 0.05 µg/kg for B1, 0.01 µg/kg for B2, 0.06 µg/kg for G1 and 0.12 µg/kg for G2, while the limits of quantification were 0.10 µg/kg for B1, 0.02 µg/kg for B2, 0.09 µg/kg for G1 and 0.21 µg/kg for G2. Values in µg aflatoxin/kg feed (ppb) obtained for the various aflatoxin fractions and the total aflatoxin content of the dog foods sampled are presented in Table 1. Aflatoxins B1, B2, G1 and G2 were detected in all the dog food brands investigated. The mean aflatoxin contents ranged from 7.76 to 11.93 µg/kg with an average of 9.61 µg/kg across the brands. The aflatoxin B1 fraction was consistently the most abundant. Details of the main constituents of the samples as indicated on the labels of the packages are presented in Table 2. All the dog food brands showed cereals as constituents of the feed. It should be noted that none of the brands showed the use of any preservatives. None of the brands also indicated expiry dates on their labels.
Table 1. Aflatoxin concentrations of dog foods sampled.

<table>
<thead>
<tr>
<th>Sample</th>
<th>B1 (µg/kg)</th>
<th>B2 (µg/kg)</th>
<th>G1 (µg/kg)</th>
<th>G2 (µg/kg)</th>
<th>Total aflatoxins (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCF</td>
<td>6.60±0.87</td>
<td>0.89±0.07</td>
<td>2.09±0.52</td>
<td>0.84±0.23</td>
<td>10.92</td>
</tr>
<tr>
<td>DTF</td>
<td>5.90±0.12</td>
<td>0.81±0.01</td>
<td>1.42±0.73</td>
<td>0.62±0.14</td>
<td>8.75</td>
</tr>
<tr>
<td>MF</td>
<td>7.14±0.15</td>
<td>1.22±0.05</td>
<td>2.60±0.82</td>
<td>0.97±0.39</td>
<td>11.93</td>
</tr>
<tr>
<td>PF</td>
<td>6.70±0.43</td>
<td>0.86±0.01</td>
<td>1.67±0.70</td>
<td>0.77±0.24</td>
<td>10.00</td>
</tr>
<tr>
<td>SP</td>
<td>5.92±0.13</td>
<td>0.80±0.01</td>
<td>1.37±0.52</td>
<td>0.70±0.21</td>
<td>8.79</td>
</tr>
<tr>
<td>TF</td>
<td>5.12±0.71</td>
<td>0.66±0.27</td>
<td>1.31±0.41</td>
<td>0.67±0.11</td>
<td>7.76</td>
</tr>
</tbody>
</table>

Table 2. Major constituents of the brands sampled.

<table>
<thead>
<tr>
<th>Feed sample/brand identification</th>
<th>Basic/main constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCF</td>
<td>Cereals (assorted grains), fish, meat, bone, maize, groundnut cake</td>
</tr>
<tr>
<td>DTF</td>
<td>Cereals (maize), fish meal, bone meal, vitamins, meat, flavor, minerals, groundnut</td>
</tr>
<tr>
<td>MF</td>
<td>Cereals (maize), fish meal, bone meal, groundnut cake, minerals</td>
</tr>
<tr>
<td>PF</td>
<td>Cereals, rice, fish meal, meat meal, bone, salt, vitamins and minerals, oil, additives: Vitamins A, D3, E, B1, B2, nacin, choline, chlorides, zinc, copper and iodine</td>
</tr>
<tr>
<td>SP</td>
<td>Cereals, indomie noodles, fishmeal</td>
</tr>
<tr>
<td>TF</td>
<td>Cereals (maize), fish meal, bone meal, groundnut cake, minerals</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Contamination of animal feed with mycotoxins, and especially aflatoxins poses serious health threats to animals. After ingestion, aflatoxins are absorbed and carried to the liver via the circulatory system. They are then converted by the liver into toxic reactive epoxides which bind covalently to intracellular macromolecules such as DNA, RNA and protein enzymes, resulting in damage to liver cells (Cullen and Newberne, 1994). The primary clinical effects in aflatoxicosis are related to hepatic damage in all species studied.

The results of this study shows that dog foods marketed in Ibadan, Nigeria are frequently contaminated with aflatoxins at levels that are considered to be capable of inflicting some level of chronic poisoning to the dogs that consume them. The aflatoxin B1 fraction was the most abundant in all the dog food brands, confirming earlier observations of aflatoxin B1 being the most prevalent fraction of the aflatoxins (Puschner, 2002; IARC, 1993).

In contrast, in a similar study carried out on 76 dry dog food samples in Vienna, Austria, none of the analyzed samples contained aflatoxin at levels above the detection limit of 0.5 µg/kg, although other mycotoxins including Deoxynivalenol, Zearalenone, Fumonisins and Ochratoxins were detected in low concentrations (Bohm et al., 2010). The result was attributed to a generally very effective aflatoxin control within the European countries. The major commodities which are known to be susceptible to aflatoxin contamination (example, peanuts and maize) are strictly controlled, particularly in imported products. The situation is different in countries where aflatoxin occurs more frequently in domestic products.

Several surveys in different countries indicate that commercial dog and cat foods tend to generally contain lower aflatoxin content when compared with those of birds, cattle and other species (Scudamore et al., 1997; Maia et al., 2002; Cullen and Hagler, 1994; Sharma and Marquez, 2001; Gunsen and Yaroglu, 2002). Although, the percentage of aflatoxin-positive samples varies by survey, almost all the positive samples contained less than 20 µg aflatoxin B1/kg of pet food (Leung et al., 2006). The results obtained in this study would also appear to conform with the trend observed in the previous surveys.

From a clinical point of view, such a low level of aflatoxin exposure appears to be insufficient to cause
noticeable symptoms in companion animals, but the chronic hepatotoxic and carcinogenic effects should not be overlooked. With regards to the clinical presentation of aflatoxicosis, it has been observed that sub-acute aflatoxicosis (0.5 to 1 mg aflatoxin/kg pet food) is characterized by anorexia, lethargy, jaundice, intravascular coagulation and death in 2 to 3 weeks (Boermans and Leung, 2007). Similar hepatotoxic effects can also be produced by chronic aflatoxin exposure with 0.05 to 0.3 mg aflatoxin/kg pet food over 6 to 8 weeks. Histopathology of animals with chronic aflatoxicoses revealed shrunken livers with extensive fibrosis (Newberne et al., 1966; Ketterer et al., 1975). Other signs that have been associated with aflatoxin poisoning include tenesmus and haemorrhagic diarrhoea. These observed clinical signs are, however, not specific for aflatoxicosis. As a result, many field outbreaks go undiagnosed (Tapia and Seawright, 1985). Leptospirosis is often wrongly diagnosed in cases of dogs presented with aflatoxicosis. Leptospirosis in dogs presents signs including icterus, depression, hemorrhages with blood-stained faeces which are essentially similar to those observed in aflatoxicosis, making the latter a possible differential diagnosis. Polymerase chain reaction (PCR) was actually used to eliminate the possibility of occurrence of leptospirosis in some dogs suspected to have aflatoxicosis (Newman et al., 2007).

It is important to note also that majority of dog cases presented with the observed signs in veterinary clinics within the study area possess up to date records of vaccination with leptospirosis vaccines which would appear to contradict the otherwise reported high prevalence of leptospirosis in the area. It is therefore, reasonable to always include chronic aflatoxin poisoning as a possible cause of these presentations as many of these dogs have started having exposures to the commercial dog foods from the time of weaning. Better equipments or veterinary facilities in the country for effective definitive diagnosis of disease conditions are thereby advocated.

Mycotoxins that adversely affect human and animal health are found mainly in post-harvest crops including cereals (corn) which constitute a major ingredient in the dog foods sampled in this study; in most cases, commercial dry dog foods marketed in Ibadan incorporate cereal grains and groundnut that have been stored for considerable period of time. Mold growth on grain under field conditions or during storage occur at moisture levels above 16% and at temperatures above freezing (Zaki et al., 2012), a condition usually obtained in the tropical humid conditions. Packaged food would also not be expected to be stored indefinitely, especially in the absence of any preservatives; the situation obtained in the dry dog foods sampled.

Regulation of mycotoxin content of animal feed worldwide mainly focuses on farm animals, with less attention on companion species (Leung et al., 2006). In most countries, pet food is regulated by a maximum mycotoxin contamination for all feedstuffs rather than pet-specific legislation. Both the United States and Canada, for example, have a 20 µg/kg legal limit for aflatoxins B1, B2, G1 and G2 for all animal feed. The Food and Drug Administration (FDA) of the United States suggests a zero tolerance for aflatoxin in food but lists a legal limit of 20 µg/kg (ppb) in dog feed (Edds, 1973; Green et al., 1977). In the European community generally, the level of aflatoxin B1 in feed ingredients is set at 10 µg/kg while countries like Turkey maintains the tolerance limit of 10 µg/kg (Gunsen and Yaroglu, 2002), some other countries like the Netherlands has decreased this limit to 5 µg/kg (Abarca et al., 1994). Switzerland and Brazil for instance, have legal limits of 10 and 50 µg/kg, respectively for aflatoxin B1 in all animal feeds (Verordnung, 1999: Brazil Ministry of Agriculture, 1988). Such tolerance limits are yet to be stipulated for many food ingredients and food products, including pet food products in Nigeria. As noted by Leung et al. (2006), however, government regulations of mycotoxin contamination appear to reflect analytical detection limits and regional prevalence as well as trade relationships among different countries, but do not necessarily represent the safe limit for mycotoxin exposure in pet animals. Levels as low as 0.05 to 0.3 mg aflatoxin/kg pet food consumed over 6 to 8 weeks can produce chronic effects on pets (Boermans and Leung, 2007).

A variety of prevention and control strategies have been suggested to minimize the risk of mycotoxin poisoning in food ingredients used in Nigeria. These include instituting a workable Hazard Analysis and Critical Control Point (HACCP) plan for each commodity; genetic breeding of mycotoxin-resistant crops; drying commodities to safe moisture level example 12% and during storage, keeping them below 0.70 water activity; cleaning grains and keeping them cool during storage to reduce insect and fungal growth; use of mould inhibitors like propionic acid and the use of binding agents (Jouany, 2007; Zaki et al., 2012). Dietary supplementation with large neutral amino acids (Huwig et al., 2001), antioxidants (Abdel-Wahhab et al., 2008) and omega-3 polyunsaturated fatty acids as well as inclusion of mycotoxin-sequestering agents and detoxifying microbes may ameliorate harmful effects of mycotoxins in contaminated pet food. Veterinarians are advised to always consider aflatoxicosis as a differential diagnosis in cases of chronic conditions presenting similar manifestations.

In conclusion, the results of this survey shows that commercial dry dog foods marketed in Ibadan, Nigeria are frequently contaminated with aflatoxins at levels that can potentially produce chronic toxicosis in dogs. It is thus, critically important to monitor pet foods for the presence of mycotoxins. Stringent control of mycotoxin contamination of the raw materials is recommended for pet food producers in preventing high financial losses.
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