A comparison of mycotoxin contamination of premium and grocery brands of pelleted cat food in South Africa

Contamination with mycotoxins is of concern to pet owners and veterinary practitioners owing to their ability to cause disease and exacerbate the pathological changes associated with other diseases. Currently, there is a lack of information regarding the mycotoxin content of common premium brand (PB) and grocery brand (GB) cat feeds. Therefore, we undertook to determine the mycobiota content of feed samples, from both categories (n = 6 each), and measured the levels of aflatoxin (AF), fumonisin (FB), ochratoxin A (OTA) and zearalenone (ZEA) by high performance liquid chromatographic analysis. There were high concentrations of mycotoxins in both categories of feed, regardless of the notion that PBs are of a higher quality. The concentration of these toxins may contribute to the development of related pathologies in felines.

Introduction

Mycotoxins have been implicated in adverse effects in both human and animal health (Fink-Gremmels 1999; Pulina et al. 2014). In a worldwide survey (2004–2011) of over 17 000 samples of feed or feed ingredients, it was found that more than 75% of samples were contaminated by at least one mycotoxin and 40% of the samples contained at least two mycotoxins (Streit et al. 2013). Currently, about 300 mycotoxins have been identified but not all are necessarily implicated in toxicity. The Food and Agriculture Organization (FAO) estimates that a quarter of the food produced globally is contaminated with mycotoxins. This causes significant economic losses as well as poses a serious threat to human and animal health (Bryden 2012; Vasanthi & Bhat 1998). Hence, regulatory limits have been recommended by organisations such as the Food and Drug Administration (FDA) for the common mycotoxins. Mycotoxins commonly implicated in and associated with animal health concerns include aflatoxin (AF), fumonisin (FB), ochratoxin A (OTA), trichothecenes and zearalenone (ZEA) (Boermans & Leung 2007).

Dry, pelleted pet food often contains 5% – 25% of animal protein or its derivatives with the remaining ingredients consisting of corn, corn gluten, wheat, wheat gluten and rice and its by-products, amongst other ‘millings’ (Klich & Pitt 1988). In a highly competitive pet food market, cost-cutting exercises are inevitable, leading to a compromise in the quality of products entering the retail sector. These cereal products that are often unfit for human consumption can act as excellent substrates for fungal proliferation and production of mycotoxins that contribute to liver, kidney and other diseases in pets (Bucci et al. 1998; Dereszynski et al. 2008). It is the contamination of cereals at harvest, post-harvest, manufacture and then storage (Bennett & Klich 2003; Tulpule 1981) that often becomes a health risk to pets by causing mycotoxicosis incidents and death. In 2011, South Africa experienced an outbreak of aflatoxicosis as a result of the consumption of poor quality, low-cost pelleted food (Arnot et al. 2012). The exacerbating factor was mouldy and low-grade peanuts that were contaminated with Aspergillus flavus and Aspergillus parasiticus.

In this study, we compared the mycotoxin profiles of premium brand (PB) and grocery brand (GB) cat food. PB products are perceived to have low amounts of cereal whilst GBs are perceived to have higher cereal content. Though no major mycotoxin outbreaks have been recorded in felines in recent years, the implication of mycotoxins and their role in feline health cannot be ignored (De Souza & Scussel 2012). Examination of cat food labelling on packaging reveals that claims of high quality, low-cost pelleted food (Arnot et al. 2012). The exacerbating factor was mouldy and low-grade peanuts that were contaminated with Aspergillus flavus and Aspergillus parasiticus.

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Materials and methods

**Materials**

Chemicals, reagents and mycotoxin standards were obtained from Merck (South Africa) and Sigma (South Africa) unless otherwise specified. All mycotoxin standards, except the fumonisin B, were purchased from Sigma (St. Louis, USA), whilst fumonisin B, (FB) and FB, were purchased from PROMEC (MRC, South Africa). For this study, PB refers to all veterinary restricted brands that may be purchased at veterinary practices or retail veterinary shops (Vetshops) only and generally are priced between R80.00 and R120.00 per kilogram, whilst GBs are commonly sold in supermarket and grocery outlets at a lower price range between R30 and R60 per kilogram.

**Methodology**

**Sampling**

Pelleted cat food (n = 12) from two marketing channels (PB and GB) were selected for this study. Samples were purchased from their respective outlets in convenient sizes of 2 kg – 3 kg packets. Information on brand, package size, expiry date and barcode serial numbers were recorded. Each packet of food was emptied into a 5-L bucket and thoroughly mixed by shaking. The sampling technique was adapted from methods described by Tittlemier et al. (2011). The bucket was divided into quadrants and approximately 125 g per quadrant sample was scooped up with a clean metal ladle. The samples were thoroughly mixed prior to obtaining a representative sub-sample of 500 g of which a further sub-sample of 200 g was taken by dividing 500 g into four sub-samples and 50 g taken from each quadrant. All feed samples (200 g each) were milled to a fine powder using a mechanical blender (Petron 3600, Germany). The milled samples were used for fungal culture and mycotoxin determination. Remaining samples were resealed and stored in sealed containers at 4 °C until required for further analysis.

**Fungal isolation:** Fungal isolation as well as subculturing and subsequent identification of fungi were done as previously described (Kaufman, Williams & Sumner 1963; Singh & Chuturgoon 2017).

**Mycotoxin extraction and clean-up of feed samples:** Mycotoxin extractions were done as described (Singh & Chuturgoon 2017). Mean recoveries are provided in Table 1.

**Thin layer chromatography:** Thin layer chromatography (TLC) was run for each mycotoxin as previously described (Singh & Chuturgoon 2017).

**High performance liquid chromatographic analysis of feed sample extracts:** High performance liquid chromatographic (HPLC) analysis of feed sample extracts was performed as previously described (Singh & Chuturgoon 2017).

**Results**

Thin layer chromatography characterisation and HPLC quantitation (µg/mL) were performed for the commonly suspected mycotoxins implicated in pet food contamination, namely, AF, FB, OTA and ZEA (Liggett et al. 1986; Shephard & Sewram 2004; Stenske et al. 2006). The most prevalent fungal isolates in all samples were Aspergillus species, Fusarium species and Penicillium species (Table 2). These fungi were found in both PB and GB feed categories. The fungal species Aspergillus flavus, Aspergillus fumigatus and Aspergillus niger were more commonly isolated while A. parasiticus, Aspergillus ochraceus, Aspergillus poae and Aspergillus penicillioides were found less commonly in the samples tested.

Using TLC, all samples in both categories tested positive for four mycotoxins (Table 3). The PB samples appeared to fare worse than GB samples, particularly in terms of AF and ZEA concentrations. HPLC analysis investigated AF for AFB, and AFB, while FB was evaluated for FB, and FB, besides OTA and ZEA. Both PB and GB failed the limits set by the Fertilizer, Farm Feeds, Agricultural Remedies and Stock remedies Act (No. 36 of 1947) of 10 ppb (1 ppb = 1 µg/L) for total AFS (South African Government 2009). The levels of AFS (Table 4).

**TABLE 1:** Mean recoveries of selected mycotoxins after spiking in feed samples using high performance liquid chromatography.

| Mycotoxin | Concentration spiked (µg/kg) | Concentration measured (µg/kg) | % of recovery |
|-----------|-----------------------------|--------------------------------|---------------|
| AFB1      | 100                         | 95.5                           | 95.5          |
| AFB2      | 100                         | 89.0                           | 89.0          |
| OTA       | 100                         | 94.6                           | 94.6          |
| ZEA       | 100                         | 93.0                           | 93.0          |
| FB1       | 200                         | 196.4                          | 98.2          |
| FB2       | 200                         | 193.0                          | 96.5          |

**TABLE 2:** Fungal species identification and selected mycotoxin detection in premium brand and grocery brand cat pelleted feed samples.

| Fungal species | Fungal sub-species | Fungal isolates (CFU's/mL) |
|----------------|-------------------|----------------------------|
| Aspergillus    |                   |                            |
| A. flavus      |                   | ***                        |
| A. fumigatus   |                   | **                         |
| A. niger       |                   | **                         |
| A. niveus      |                   | **                         |
| A. ochraceus   |                   | **                         |
| A. parasiticus |                   | **                         |
| A. penicillioides |              |                            |
| A. poae        |                   | **                         |
| Fusarium       |                   |                            |
| F. gramineanum |                   | **                         |
| F. verticillioides |              |                             |
| Penicillium    |                   |                            |
| Penicillium spp.|                 | **                         |
| P. polonincum  |                   | **                         |
| P. crustosum   |                   |                            |
| Other          |                   |                            |
| Rhizopus spp.  |                   | +                          |
| Unidentified microbe |    | +                          |
| Yeast          |                   | +                          |

A., Aspergillus; F., Fusarium; P., Penicillium.
*100–300 x 10^4 CFU; **, 300–500 x 10^4 CFU; ***, > 500 x 10^4 CFU; +, positive only.

**TABLE 3:** The results of thin layer chromatography characterisation.

| Mycotoxin           | TLC characterisation |
|---------------------|----------------------|
|                     | Premium              | Grocery              |
| Aflatoxin           | ***                  | ***                  |
| Fumonisin           | *                    | **                   |
| Ochratoxin A        | **                   | **                   |
| Zearalenone         | **                   | *                    |

TLC, thin layer chromatography.
***, very intense spot; **, intense spot; *, spot (intensity of spot as compared to a standard of each mycotoxin).
detected in the PB were over the set limits for both AFB$_1$ (125.02 ppb) and AFB$_2$ (11.77 ppb) but GB only exceeded the limits for AFB$_1$ (41.57 ppb). The amounts of both AFB$_1$ ($p = 0.0087$) and AFB$_2$ ($p = 0.0091$) in PB were statistically significantly higher as compared to GB. *Fusarium graminearum* was predominantly isolated in both categories; however, HPLC analysis indicated that the GB had exceedingly high concentrations of both FB$_1$ (202.53 ppb) and FB$_2$ (118.37 ppb), failing the limit set by the Food and Drug Administration of 100 ppb (FDA 2001). The amounts of both FB$_1$ ($p = 0.028$) and FB$_2$ ($p = 0.0041$) were significantly higher in GB as compared to PB. OTA ($p = 0.0196$) and ZEA ($p = 0.0060$) levels were significantly higher in PB as compared to GB (Table 4).

In summary, the PB fared worse than the GB in its AF, OTA and ZEA contamination, whilst GB contained much higher levels of FB than PB.

**Discussion**

Cats are obligatory carnivores and require taurine in their diets. A good animal protein source will provide the taurine required for a cat’s good health. The presence of high amounts of mycotoxins in commercial cat diets is indicative of high cereal content. PBs are perceived as better quality feeds, but their cereal content makes them susceptible to mycotoxin contamination. Many researchers have reported the simultaneous occurrence of several mycotoxins in feed and feed ingredients (Fox, Hodgkins & Smart 2012; Mwanza 2007; Tulpule 1981). This potent mycotoxin combination may result in synergistic action and potentiate effects that support the multi-aetiological theory (Boermans & Leung 2002).

Irrespective of marketing channels, all products were contaminated with mycotoxins. The mean AF concentration across the various brands indicates that all products failed the prescribed limit (10 ppb; by the *Fertilizer, Farm Feeds, Agricultural Remedies and Stock remedies Act* [No. 36 of 1947]; South African Government 2009). The long-term exposure of cats to mycotoxins may be implicated in numerous clinical conditions such as neoplasia, reduced immunity and poor growth and fertility (De Souza & Scussel 2012).

**Conclusion**

PBs are marketed as superior feeds, but their cereal content makes them susceptible to mycotoxin contamination. Many PBs are imported and the higher mycotoxin content may be attributed to lengthy transport in containers on the high seas and high humidity. Though cats may appear to be less susceptible to mycotoxicosis, the risk of long-term exposure to mycotoxins coupled with poor health or concurrent disease could result in increased susceptibility. Further *in vivo* studies are required to evaluate feline susceptibility to mycotoxins.

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Competing interests
The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors’ contributions
S.D.S. performed experiments, analysed data and prepared draft manuscript. S.B. assisted with analyses and preparation of the manuscript. A.A.C. was the supervisor of S.D.S. for a PhD and assisted with data analysis and drafting of the final manuscript.

References
Antonissen, G., Martel, A., Pasmans, F., Ducatelle, R., Verbrugge, E., Vandenbroucke, V. et al., 2014, ‘The impact of Fusarium mycotoxins on human and animal host susceptibility to infectious diseases’, Toxins 6, 430–452. https://doi.org/10.3390/toxins60200430

Arnot, L.F., Duncan, N.M., Coetzer, H. & Botha, C.J., 2012, ‘An outbreak of canine aflatoxicosis in Gauteng Province, South Africa’, Journal of the South African Veterinary Association 83(1), Art. no. 2, 1–4. https://doi.org/10.4102/jsava.v83i1.2

Bennett, J. & Klich, M., 2003, ‘Mycotoxins’, Clinical Microbiology Reviews 16, 497–516. https://doi.org/10.1128/CMR.16.4.497-516.2003

Boerma, H.J. & Leung, M.C., 2007, ‘Mycotoxins and the pet food industry: Toxico logical evidence and risk assessment’, International Journal of Food Microbiology 119, 95–102. https://doi.org/10.1016/j.ijfoodmicro.2007.07.063

Bryden, W.L., 2012, ‘Mycotoxin contamination of the feed supply chain: Implications for animal productivity and feed safety’, Animal Feed Science and Technology 173, 134–158. https://doi.org/10.1016/j.anifeedsci.2011.12.014

Bucci, T.J., Howard, P.C., Tolleson, W.H., Laborde, J.B. & Hansen, D.X., 1998, ‘Renal effects of fumonisin mycotoxins in animals’, Toxicologic Pathology 26, 160–164. https://doi.org/10.1080/01926233.1998.9695005

Creppy, E.E., Chiarappa, P., Baurdrom, I., Borracci, P., Mouha, S. & Carrato, M.R., 2004, ‘Synergistic effects of fumonisin B1 and ochratoxin A: Are in vitro cyto toxicity data predictive of in vivo acute toxicity?’, Toxicology 201, 115–123. https://doi.org/10.1016/j.tox.2004.04.008

Deresynski, D.M., Center, S.A., Randolph, J.F., Brooks, M.B., Hadden, A.G., Palayda, K. et al., 2008, ‘Clinical and clinicopathologic features of dogs that consumed foodborne hepatotoxic aflatoxins: 72 cases (2005–2006)’, Journal of the American Veterinary Medical Association 232, 1329–1337. https://doi.org/10.2460/javma.232.9.1329

De Souza, K.K. & Sussel, V.M., 2012, ‘Occurrence of dogs and cats diseases records in the veterinary clinics routine in South Brazil and its relationship to mycotoxins’, International Journal of Applied Science and Technology 1(8), 129–134.

D’Mello, J., Placinta, C. & Macdonald, A., 1999, ‘Fusarium mycotoxins: A review of global implications for animal health, welfare and productivity’, Animal Feed Science and Technology 80, 183–205. https://doi.org/10.1016/S0377-8401(99)00059-0

Fink-Gremmels, J., 1999, ‘Mycotoxins: Their implications for human and animal health’, International Journal of Food Science and Technology 34, 9623–9635. https://doi.org/10.1022/jifs.6363

Gigget, A., Colvin, B., Beaver, R. & Wilson, D., 1986, ‘Climine aflatoxicosis: A continuing problem’, Veterinary and Human Toxicology 28, 428–430.

Hocking, A., Holds, K. & Tobin, N., 1988, ‘Intoxication by tremorgenic mycotoxin Penicillium crustosum from rice’, Canadian Journal of Veterinary Research 52, 1–5.

Ketterer, P., Williams, E., Blaney, B. & Connole, M., 1975, ‘Climine aflatoxicosis’, Australian Veterinary Journal 51, 355–357. https://doi.org/10.1111/j.1751-0813.1975.tb15946.x

Klich, M. & Pitt, J., 1988, ‘Differenciation of Aspergillus flavius from A. parasiticus and other closely related species’, Transactions of the British Mycological Society 91(1), 99–108. https://doi.org/10.1016/S0007-1536(88)80010-5

Leung, M.C., Diaz-Llano, G. & Smith, T.K., 2006, ‘Mycotoxins in pet food: A review on worldwide prevalence and preventative strategies’, Journal of Agricultural and Food Chemistry 54, 9623–9635. https://doi.org/10.1021/jf063263h

Mwanza, M., Nduw, R.V., Dzoma, B., Nyirenda, M. & Bakunzi, F., 2013, ‘Climine aflatoxicosis outbreak in South Africa (2011): A possible multi-mycotoxins aetiology’, Journal of the South African Veterinary Association 84(1), Art. no. 193, 1–5. https://doi.org/10.4102/jsava.v84i1.133

Naudé, T., O’Brien, O., Rundberget, T., Megregor, A., Roux, C. & Flåyven, A., 2002, ‘Tremorgenic neurotoxicosis in 2 dogs acissed to the ingestion of penitrem A and possibly roquefortine in rice contaminated with Penicillium crustosum’, Journal of the South African Veterinary Association 73, 211–215. https://doi.org/10.1016/j.jsva.2010.04.006

Patterson, D. & Roberts, B., 1979, ‘Mycotoxins in animal feedstuffs: Sensitive thin layer chromatographic detection of aflatoxin, ochratoxin A, sterigmatocystin, zearalenone, and T-2 toxin’, Journal of the Association of Official Analytical Chemists 62, 1265–1267.

Placinta, C., D’Mello, J. & Macdonald, A., 1999, ‘A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins’, Animal Feed Science and Technology 78, 21–37. https://doi.org/10.1016/S0377-8401(98)00027-6

Pullina, G., Battagone, G., Brambilla, G., Cheli, F., Danelli, P.P., Masero, F. et al., 2014, ‘An update on the safety of foods of animal origin and feeds’, Italian Journal of Animal Science 13, 3571. https://doi.org/10.4081/ijas.2014.3571

Ryu, D., Jackson, L.S. & Bullerman, L.B., 2002, ‘Effects of processing on zearalenone’, Advances in Experimental Medicine and Biology Series 504, 205–216. https://doi.org/10.1007/978-1-461-46029-4_21

Sheppard, G. & Sewram, V., 2004, ‘Determination of the mycotoxin fumonisin B1 in maize by reversed phase thin-layer chromatography: A collaborative study’, Food Additives and Contaminants 21, 498–505. https://doi.org/10.1080/0265203041001670175

Singh, S.D. & Chuturgoon, A.A., 2017, ‘A comparative analysis of mycotoxin contamination of supermarket and premium brand pelleted dog food in Durban, South Africa’, Journal of South African Veterinary Association 88, a1488. https://doi.org/10.4102/jsava.v88i1.1488

South African Government, 2009, Fertilizers, farm foods, agricultural remedies and stock remedies act (Act No.36 of 1947), South African Government Gazette No. R 2227, 2009, March 06, Government Printer, Pretoria.

Stenske, K.A., Smith, J.R., Newman, S.J., Newman, L.B. & Kirk, C.A., 2006, ‘Aflatoxicosis in dogs and dealing with suspected contaminated commercial foods’, Journal of the American Veterinary Medical Association 228, 1686–1691. https://doi.org/10.2460/javma.228.11.1686

Street, E., Naehrer, K., Rodrigues, I. & Schatzmayr, G., 2013, ‘Mycotoxin occurrence in feed and feed raw materials worldwide: Long-term analysis with special focus on Europe and Asia’, Journal of the Science of Food and Agriculture 93, 2892–2899. https://doi.org/10.1002/jfsa.6225

Tuttleman, S., Varga, E., Scott, P. & Kriska, R., 2011, ‘Sampling of cereals and cereal-based foods for the determination of ochratoxin A: An overview’, Food Additives and Contaminants 28, 775–785. https://doi.org/10.1080/19440049.2011.559278

Tulpule, P., 1981, ‘Aflatoxicosis – Experimental studies’, Journal of Cancer Research and Clinical Oncology 99, 137–142. https://doi.org/10.1007/BF00434449

Vazanthi, S. & Bhat, R.V., 1998, ‘Mycotoxins in foods occurrence, health & economic significance & food control measures’, Indian Journal of Medical Research 108, 212.

Young, K.L., Villar, D., Carson, T.L., Ierman, P. & Bottoff, M.R., 2003, ‘Tremorgenic mycotoxin intoxication with penitrem A and roquefortine in two dogs’, Journal of the American Veterinary Medical Association 222, 52–53, 35.