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Determination of Aflatoxins in Commercial Dog Foods by Immunoaffinity Column

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Abstract: The occurrence of aflatoxins B_1, B_2, G_1 and G_2 in commercial dog food was investigated. Fourteen dog food cereals-based from dog shops were collected. The extracts were cleaned- up through commercially immunoffinity columns followed by fluorescence detection and pH analyzed. Dry and semi-dry adult food showed pH between 6.0-6.2 in average aflatoxins 24 and 17.5 μg kg^{-1}, respectively. The dry and semi-dry puppy food with pH (6.2- 6.8) and levels ranged from 17-29 μg kg^{-1}; three semi-dry adult food with a pH>6. The 42.85% of total foods were above the maximum limit established in Mexico. These results show the occurrence of aflatoxins, indeed the regulatory aspects still need harmonized; also the importance of pH in the performance of immunoaffinity column.

Key words: Pet food, immunoaffinity columns, aflatoxinas, aflatoxicosis, regulation

INTRODUCTION

The commercial food market for pets in Mexico registers 84 firms, some nationals and importing in its majority, cereal grains are often used as ingredients in formulation of pet food, they may contain mycotoxins, in spite of the situation there's a little information on the occurrence of aflatoxins.

The aflatoxins are chemical molecules derived from difuranocumarinas, they are produced by different species of *Aspergillus sp*., which may grow on a variety of crops including corn, rice, wheat (Maxwell *et al*., 2006), being dry those of greater content in grains. Aflatoxins are hepatotoxic and carcinogenic according to Mwanda (2005).

The maximum level accepted in feed of aflatoxins is 20 μg kg^{-1} in USA, Canada and Mexico; Europe established 10 μg kg^{-1} and Brazil admits 50 ppb (Creppy, 2002). In 1999, on Texas state died 25 dogs by commercial pet food consumption contaminated with fungi and mycotoxins. In 2001, on Mexico aflatoxins were detected at 89% of the dog food samples which mean values of 5 μg kg^{-1}. Maia *et al*. (2002), in Brazil detected at 12% of commercial foods for dogs contaminated with aflatoxinas. In 2005, a serious problem in Venezuela was provoke, by the contamination of aflatoxinas in foods for dogs and cats, causing the death in several animals and the exit of the market of foods; in 2006, 100 dogs wee dead in outbreaks in New York, South Carolina, North Carolina, Georgia, Massachusetts, Ohio and Pennsylvania in the United States. On April, of this year, the Food and Drug Administration (FDA) provided on update on the recall on contaminate pet foods (Stenske and Smith, 2006; Smith *et al*., 2007).

Various analytical methods have been developed for the reliable detection of aflatoxinas in animal foods (Maxwell *et al*., 2006). The immunoassay method belongs to selective, sensitive due to columns that they are prepared by binding antibodies specific for mycotoxins. They are fast techniques, relatively inexpensive, although there are concerns that may affect the accuracy of analysis (Castegnaro, 2006). The immunoaffinity columns are detection limits between 5-300 ppb (Aflatest *et al*., 2000).

Regulation of mycotoxins content of animal feed worldwide mainly focuses on farm animals, with less attention to pet species. USA, Canada and Mexico have a 20 μg kg^{-1} limit for aflatoxinas, but with enormous variations between countries (10-50 μg kg^{-1}). However, the aflatoxins are highly carcinogenic and there isn't any dose (Newing *et al*., 2000).

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This study was made to find out the level of aflatoxins in importing commercially dog food as well as their risk to animal health.

MATERIALS AND METHODS

The study was carried out in the city of Mexico, in June to September of 2003. Fourteen commercial food samples were collected at random from pet shops. Three dry foods and eleven semi-dry food. The total samples were ground and weigh to obtain analytical sample of 100 g. Each sample of 50 g was extracted by the mixture of methanol and water mixture (80: 20 v v^{-1}) and registry pH by potenciometry method AOAC. The immunoaffinity columns containing monoclonal anti aflatoxin antibodies (Aflatest TM, Vicam were used to cleaned-up the extracts and quantified by fluorescence detection after reaction with bromine solution. The fluorometer was calibrate with standars of aflatoxins purchase to Vicam. The long wavelongth UV filter, 450 nm excitation filter and 415 nm emission filter to measure fluorescence. The results are redding in ppb. Data from were analyzed by one-way analysis of mean, range, standard and coefficient of variation.

Table 1: Results of pH and aflatoxins detected by immunoassay with fluorometry in commercial dog foods

Food	pH	Aflatoxins (ppb)
Dry adult	5.5	21
Dry puppy	6.2	17
Dry adult	6.5	27
Semi-dry puppy	7.0	27
Semi-dry puppy	6.7	31
Semi-dry adult	7.0	15
Semi-dry adult	6.0	14
Semi-dry adult	6.8	20
Semi-dry adult	6.5	25
Semi-dry adult	2.5	6
Semi-dry adult	4.0	4
Dry adult	4.9	9
Dry adult	6.0	15
Dry adult	5.3	16

Table 2: Comparision of results of aflatoxins contamination in dog foods with the values accepted regulation in Mexico

Values of aflatoxins in dog food commercialized in Mexico	No of foods detected with a maximum of aflatoxins accepted	No food detected with a minimum levels of aflatoxins accepted
21	21	21
17	17	17
27	27	
27	27	
31	31	
15	15	15
14	14	14
20	20	
25	25	
6	6	6
4	4	4
9	9	9
15	15	15
16	16	16

Levels minimum (20) and maximum (50 ppb) of aflatoxins accepted in different countries

Table 3: Mean, standard error, range and coefficient of variation of the results from the aflatoxins analysis of commercial foods for dogs (n = 14)

Foods	Range	Mean	Standard error	Coefficient of variation
Dry foods	18	17.50	3.43	19.60
Semi dry foods	27	17.75	2.97	16.73

Significance level (p = 0.95), R^2 = 0.000252, There was not difference among food in relation to the aflatoxins content

RESULTS

The results of the study are summarized in Table 1. The dry adult food showed pH = 6.0 and aflatoxins in average 24 μg kg^{-1}; in puppy food a pH = 6.2 and 17 ppb aflatoxins. In semi-dry adult food a pH = 6.2 and 17.5 μg kg^{-1} and semi-dry puppy food a pH = 6.8 and 29 μg kg^{-1}. Only three canned adult food has a pH = 4.1 and 6.1 ppb. Levels of aflatoxins B_1+B_2+G_1+G_2 above the maximum limit established (Table 2) in Mexico (20 μg kg^{-1}) were detected in six of 14 positive samples (42.85%) in dry adult food and semi-dry puppy food. If the limit of 50 μg kg^{-1} is applied then all the samples are inside, but eleven of fourteen samples (78.58%) exceeded the maximum limit proposed of Europe (10 μg kg^{-1}). In this study, the dry food were the most contaminated with aflatoxins. The maximum level of aflatoxins founded was 31 μg kg^{-1}.

DISCUSSION

The aim of study was to detected the aflatoxins contamination in dog foods commercialized in Mexico, after the results they were compared with the different levels accepted by aflatoxins, in different countries, The data has indicate that 47% of the dog foods in the Mexican market has to be considerated as unsafe for pets consumption as they contain aflatoxins above the Mexican permissible limit (20 ppb). Mould deterioration during transit has been recognized as a mayor problem leading to aflatoxins contamination (Vasanthi *et al*., 1998).

This information due be take account for the food pets industry (Freeman and Micheal, 2001). If a need a level exposure 60 μg kg^{-1} of aflatoxins for signs of liver dysfunction in dogs all this samples could be able to cronic aflatoxicosis with possibly a atypia of hepatocytes. It is not easy to define a mycotoxicosis when the diagnosis depends upon mycological identification of a species rather than on identifying the mycotoxin itself (Bueno and Silva, 2001). The pH is a physical-chemic parameter of quality, the two semi-dry food were pH > 4.6, probably means a deterioration of ingredients grains and meals into the foods (Ahlstrom and Krogdahl, 2004).

The sensitivity limit of the immunoaffinity columns is 10 μg kg^{-1} of aflatoxins, the dog foods detected under can not be considered like positive reliable, since in addition they samples presented the greater acidity (pH

of 2.5, 4.1, 4.9) what could interfere with in the efficiency of the column. According to Castegnaro *et al.* (2006), indicated the necessity to validate the method using immunoaffinity columns for each complex matrix.

CONCLUSION

In conclusion, analysis of feed for aflatoxins consistently revealed the presence of aflatoxins, the levels raged from 4- 31 ppb. The most contaminated commercial dog food were the puppy semi-dry food, reason why is necessary to survey of aflatoxins contamination in the chain foods for pets. The homologation of the tolerable limits for aflatoxins in pet food is recommended. The pH is a good parameter to evaluate the deterioration of samples. Thus, the performance of immunoafinity column is necessary to each food. There was not difference among food in relation to the aflatoxins content.

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