

CONSTRUINDO SABERES, FORMANDO PESSOAS E TRANSFORMANDO A PRODUÇÃO ANIMAL

AFLATOXINS DETERMINATION IN COMMERCIAL DOG FOOD

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Resumo: Aflatoxinas são metabolitos produzidos por diferentes tipos de fungos (*Penicillium sp*, *Aspergillus sp*, *Fusarium sp*), que podem ser tóxicos para diferentes espécies animais e até para humanos. Para se determinar a presença de aflatoxinas em rações comerciais para cães, vendidas e comercializadas na cidade de Toluca, México, 20 amostras foram coletadas e processadas através da cromatografia de camada fina (teste qualitativo), com o método Stoloff. Os resultados foram analisados através de estatística não paramétrica. A ocorrência positiva de 80% foi obtida para aflatoxinas do tipo G1 e G2. A ingestão dessas aflatoxinas podem causar envenenamento crônico e agudo nas espécies que circunstancialmente consomem alimento contaminado, incluindo humanos, proporcionando problemas nos rins, fígado, imunodeficiência em órgãos reprodutivos dentre outros. Desta forma, os riscos de contaminação durante o tratamento devem ser monitorados durante o processo de produção, tão quanto durante a manipulação e distribuição dos produtos.

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Palavras Chave: AFG1, AFG2, micotoxinas, ração para cães

Abstract: Aflatoxins are metabolites produced by different kinds of fungi (*Penicillium sp*, *Aspergillus sp*, *Fusarium sp*), which can be toxic for different animal species and even for humans. In order to determine the presence of aflatoxins in commercial dog food, sold and marketed in Toluca City, Mexico, 20 samples were collected and processed through thin layer chromatography (qualitative test), with the Stoloff method. Results were expressed through non-parametric statistics. A positivity of 80% was obtained for aflatoxins G1 and G2. The intake of these aflatoxins can cause chronic and acute poisoning in the species that circumstantially consume contaminated food, including humans, producing problems on kidneys, liver, immunodeficiency and reproductive organs among others. Therefore, the risks of contamination during the treatment must be assessed and limited during the production process, as well as during the handling and distribution of food.

Keywords: AFG1, AFG2, mycotoxins, dog food

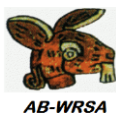
Introduction

Mycotoxins are fungal metabolites whose ingestion, inhalation or cutaneous absorption produces pathological alterations or cause the death of animals (without excluding birds) and people. They are polyketone compounds, produced by some fungi of the genera *Aspergillus*, *Penicillium* and *Fusarium* resulting from the condensation reactions produced in certain physical, chemical and biological conditions when the reduction of ketone groups in the biosynthesis of fatty acids is interrupted (Gimeno, 2007).

The most important mycotoxins in veterinary medicine are: aflatoxins (B1, B2, G1 and G2), citrinin, diacetoxyscirfenol, ochratoxin, oxalic acid, rubratoxin, trichothecenes, penitren A and zearalenone, among others. The degree of importance that the presence of fungal metabolites can have in food, is related to its toxicity, the nature and economic impact of the contaminated products, its proportion in humans and animals diet, the type of toxic effect produced and the possibility that this is cumulative with serious long-term effects, such as oncogenesis or mutagenesis. Exposure to mycotoxins can produce both, acute and chronic toxicity, with results ranging from death to harmful effects on the central nervous system, cardiovascular, respiratory and digestive systems; they are also carcinogenic, teratogenic and immunosuppressive agents (FAO, 2005).

Currently dog breeding has being intensified both in commercial breeding sites as well as in homes, adopting them as favorite pets. So this species, carnivorous in nature, have been adapting to the food change that man has imposed over them, and currently there is available a wide catalog of balanced feed of different brands. These foods are made from corn flour; it has been reported that corn, like all cereals, is susceptible to contamination by fungi and their metabolites: aflatoxins. The dog, like any other domestic species, is susceptible to aflatoxin poisoning that could be present in the food they eat, putting their health at serious risk. The reported cases of death from aflatoxicosis are usually associated with very high levels of aflatoxin

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contamination (100-6700 ppb). However, deaths with low levels of aflatoxin (13-91 ppb) have also been reported (Guzmán, 2010). In addition, the proper procedures for preparation of pet food, do not reduce or eliminate the presence of aflatoxins in raw materials. Therefore, the objective of this study was to evaluate commercial dog foods and determine if there is aflatoxin contamination that may represent an important factor of direct affection and other pathologies in dogs.

Material and methods

A random collection of 20 samples of different brands of commercial dog food was carried out in self-service centers located at Toluca City, at the central Valley of Mexico. For the extraction of mycotoxins, the method of Stoloff (1999) was used; performing three repetitions per sample. This extraction technique is characterized by using highly volatile solvents, causing a separation and dissolution of metabolites (mycotoxins), which are related to the biochemical - structural reaction with the substances used. The mixture to be analyzed is placed 1.5 cm away from the bottom edge of the plate and is introduced into a chromatographic tank, containing the mobile phase. The mobile phase rises along the plate by capillary action, displacing the components of the mixture at different speeds, causing their separation. The detection is carried out by fluorescence with an excitation wavelength of 365 nm and emission of 435 nm. The chromatogram of the aflatoxin standard and the test sample (retention times) is compared. The results obtained are expressed through descriptive statistics.

Results and discussion

Out of the 20 samples assessed, based on visual appearance (physical characteristic) and odor, none of them showed to have any abnormality. However, through the qualitative thin layer chromatography test, by means of the Stoloff method, a positivity of 80% (16/20), G1 and G2 was detected (Table 1).

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Table 1. Number of dog food samples and type of detected aflatoxin

Sample number	Stage	B1	B2	G1	G2
1	No data	-	-	+	+
2	Adult	-	-	+	+
3	Adult	-	-	+	+
4	Adult	-	-	+	+
5	No data	-	-	+	+
6	No data	-	-	+	+
7	Adult	-	-	-	-
8	No data	-	-	+	+
9	No data	-	-	-	-
10	Adult	-	-	-	-
11	No data	-	-	+	+
12	Adult	-	-	+	+
13	Adult	-	-	+	+
14	Adult	-	-	+	+
15	Adult	-	-	-	-
16	No data	-	-	+	+
17	Adult	-	-	+	+
18	Adult	-	-	+	+
19	No data	-	-	+	+
20	No data	-	-	+	+
Total		0	0	16	16

According to the methodology used, which is considered to have a high sensitivity (88%) and specificity (99%), AFG1 and AFG2 were positive, which according to Peña *et. al.* (2013), are most frequently found, contaminating different food ingredients, and that can grow and proliferate in different grains, even in the already processed food.

It has been established that various pathological problems associated to mycotoxins are characterized by species; the main ones are hepatotoxic, nephrotoxic, bone marrow affection and erythrocytes, carcinogenic, and reproductive dysfunction. Nevertheless, when associated to secondary problems, mycotoxins can cause the death of the individuals, who consume them in a constant or frequent manner. Based on the availability and consequent contamination of food, it is

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feasible that dogs that consume contaminated food can develop diverse pathologies, and without an accurate and effective diagnostic, it often can be confused with other types of diseases, unfortunately, most of the time they are underdiagnosed (Guzmán, 2010).

During the different processes of production, processing and sale of commercial foods, it is feasible that contamination occurs (physical, chemical and biological). Results obtained in our qualitative study are of much importance, since it makes evident that food management must be done with extra care, in order to reduce the risk of poisoning. Up to this moment, as far as we are concerned, there are no similar works to compare our results. However, the minimum acceptable limits of aflatoxins, for animal consumption are not yet standardized; for example, FAO does not accept levels higher than 2 ppb in prepared foods. Moreover, in Mexican legislation, "acceptable" limits for the presence of aflatoxins, still does not consider dog food and other companion animals' food, in order to determine the minimum aflatoxin values that may affect them (Rezaei, 2014).

Apart from the handling and production of aflatoxin metabolites, it is important to consider the quality of the ingredients that are been used for the dog food preparation; since aflatoxins can use certain substrates like pectins, carbohydrates in the form of polysaccharides, organic acids, proteins and lipids to develop. Some studies have shown that nutrients and the type of substrate are important for the production of aflatoxins, which can be stimulated by a high carbohydrates levels and a low level of proteins. Carbohydrates provide two carbons precursors for the synthesis of the toxin. Aflatoxins interaction with dietary lipids can cause digestive disorders, due to the decrease in digestive enzymes activity, which is expressed as a malabsorption syndrome (Gimeno, 2007).

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Conclusion

From the 20 assessed samples, a high positivity of 80% (16/20) was detected for AFG1 and AFG2; which is considered as a high percentage of aflatoxin contamination. Especial attention should be given to the dog food manufacturing process, as well as the type of ingredients that are been used. Meanwhile, specific studies to determine the maximum levels of aflatoxins that should be allowed for dog food, still need to be done and established in Mexican rules and legislation for animal feeds processing.

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