

STUDIES ON THE OCCURRENCE OF SOME MICROBIAL TOXINS IN PET FOOD

By

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ABSTRACT

A total of one hundred dog and cat food, fifty of each (25 dry and 25 canned) were analyzed for determination of their content of total aflatoxin using Enzyme linked Immunosorbent Assay (ELISA) and Staphylococcal enterotoxins using Set-RPLA. The mean value of total aflatoxin in dry food were 0.3212 ± 0.02437 and 0.3068 ± 0.01323 for dog and cat, but not detected in all examined canned pet food samples as well as Staphylococcal enterotoxins. The level of positive samples compared with maximum legal limit set by Food and Drug Administration FDA (2001) 20 ug/kg. The public health significance of aflatoxin and measure of control were discussed.

INTRODUCTION

Dog and cat food are prepared with vegetables and/or meat (chicken, beef, turkey and fish), cereal grains (maize, sorghum and rice), fat, vitamins and minerals. During its manufacturing, food can be contaminated with mold spores, especially when cereal grains are ground and food is granulated **Suárez, (1999)**. The term “companion animal” implies the existence of a strong human–animal bond between pets and their owners **Adams *et al.* (2004)**. A pet is often regarded as a family member by its owner. Pet interactions and ownership have been associated with both emotional and physical health benefits **Milani (1996); Adams *et al.* (2004)**. The human-animal bond has resulted in over sixty-four million American households in owning one or more pets, thereby creating a huge market for the pet food industry **APPMA (2006)**. Dogs and cats continue to be the most popular pet to own. Mycotoxin production is favored by high humidity, high water activity and has been detected in many agricultural crops. The mycotoxins are a highly varied group of chemical substances. When they are present in food, those may become causes of acute and/or chronic adverse health effects in animals and humans and may affect many target organs and systems, particularly liver, kidneys, nervous, endocrine and immune systems. The low levels of exposure can cause the

occurrence of chronic effect. According to Mycotoxin Survey Program, 2011 the pollution with aflatoxins, was 27 %, of the total analyzed 4327 samples collected worldwide. In terms of exposure and severity of chronic diseases (especially cancer), the mycotoxins pose a higher risk than anthropogenic pollutants, pesticides and food additives. Some mycotoxins have two carcinogenic properties - property to initiate and property to promote the carcinogenesis. Other mycotoxins seem to have mostly tumor-promoting effects. Aflatoxins are naturally occurring highly toxic mycotoxins that are produced as secondary metabolites of several species of Aspergilli, including *A.flavus* and *A.parasiticus*, *A. nomius*, *A. pseudotamarii*, *A.bombycis* **Ghada et al. (2014)**. 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 **IARC (1993); Puschner (2002)**. 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. 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. After ingestion, aflatoxins are absorbed and carried to the liver via the circulatory system. The primary clinical effects in aflatoxicosis is related to hepatic damage in all species studied. In acute aflatoxicosis, dogs exposed to 0.5-1 mg aflatoxin/kg body weight (BW) typically die within days, showing enlarged livers, disseminated intravascular coagulation and internal hemorrhaging **Bohn and Razzai - Fazeli, (2005)**. Sub-acute aflatoxicosis (0.5-1 mg aflatoxin/kg pet food) is characterized by anorexia, lethargy, jaundice, intravascular coagulation and death in 2-3 weeks. Similar hepatotoxic effects can also be produced by chronic aflatoxin exposure with 0.05-0.3 mg aflatoxin/kg pet food over 6-8 weeks. Staphylococcal food poisoning is one of the most common foodborne illnesses and results from growth of certain strains of staphylococci in poorly handled or stored foods. If staph get into food and the food is kept at improper temperatures, the bacteria can grow. If the strain of staph that's in the food is one

that can produce enterotoxins, these toxins can accumulate in the food at high enough levels to cause food poisoning when eaten. In most cases, people are probably the origin of enterotoxin-producing staph that contaminates food, but pets are another possible source. In humans and animals, staphylococci constitute part of the normal microbial flora. There are several different definitions of what constitutes an animal's normal bacterial flora. There are the "symbionts", which benefit themselves and the host; "commensals", which do not benefit the host but are harmless; and the "opportunists"—typically non-pathogenic microorganisms that act as a pathogen in certain circumstances **Sorum, and Sunde (2001)**. According to the aforementioned definitions, *Staphylococcus aureus* could be classified as an opportunistic pathogen for both humans and animals. Although the bacteria are found in clinically healthy individuals, they can cause a wide range of infections when the immune system becomes compromised or select comorbidities are associated (congestive heart failure, diabetes, pulmonary disease and renal failure) **Oliveira et al. (2002) and McKinnell et al. (2013)**. The composition of normal flora on an organism depends on the species, feed and environment, including population density. Nevertheless, *S. aureus* is the most frequently isolated coagulase positive *Staphylococcus* (CPS) from the anterior nares and temporarily from the skin of humans, whereas coagulase negative staphylococci (CNS), mainly *Staphylococcus epidermidis*, are dominant on the skin, Otto (2010). Similarly, for cats CNS are the major species in this tissue and mucosa's natural flora. The most frequently observed is *S. felis*, and seldom CPS such as *S. pseudointermedius*, but in some environments *S. aureus* is also observed **Lilenbaum (1998)**. Several methods can be used for aflatoxin and staph enterotoxin detection, such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and enzyme linked immunosorbent assay (ELISA). The later one is simple, specific, sensitive, low cost and rapid screening method (Turner et al. 2009). The aim of this study was to determine the total aflatoxin (B1, B2, G1, and G2) and staph enterotoxin content in dry and canned pet animal food by using ELISA technique.

MATERIAL AND METHODS

a. Collection of samples.

A total of hundred dog and cat food, fifty of each (25 dry and 25 canned) randomly collected from supermarkets and transferred to the laboratory where they analyzed for determination of their content of total aflatoxins and staphylococcal enterotoxin.

b. Method.

Detection of Aflatoxin residue:

The quantitative analysis of total aflatoxins was determined through a competitive direct Enzyme linked Immunosorbent Assay (ELISA) method **Mankeviciene et al., (2006)**. The method based on the accurate monitoring of mycotoxins and is suitable for screening large number of samples.

The veratox test kits (NeogenCorp. Lansing, MI. USA (certificate No 950702) and they were used for the analysis. The analysis was done according to the manufacturer’s instructions.

Detection of Staphylococcus aureus enterotoxins:

The technique was applied as **Olempiaet.al. (2006)**. for toxin detection kits of set - RPLA (Staphylococcal enterotoxins A, B, C, and D detection kits, **Oxoid (2014)**.

RESULT

Table (1): Mean ± SE of Total Aflatoxin and *S. aureus* enterotoxins in Pet Food (Dog and Cat).

Item		Total No. of Samples	Total aflatoxin residues (ppb)*	<i>S. aureus</i> enterotoxins
Dog food	Dry	25	0.3212 ± 0.02437	ND
	Canned	25	ND	ND
Cat food	Dry	25	0.3068 ± 0.01323	ND
	Canned	25	ND	ND

Ppb = part per billion = ug/kg* Mean± SE = Mean ± Standard Error for positive samples.

ND: not detected

DISCUSSION

There are three basic forms of commercial pet foods: dry, semi-moist, and moist or canned. The main difference in this categorization scheme is based on the water content of the food with dry foods containing usually less than 11% water, semi-moist foods containing 25 to 35% water, and moist or canned food containing 60 to 87% water **Zicker, (2008)** commercially prepared pet foods are easy and economical way to fulfill the nutrient requirements in pets. Our study carried on dry and canned pet foods. The Statistical Analytical Results of Total Aflatoxin residues (ppb) in dry food samples of dogs and cats were recorded in (Table 1). The mean value was 0.3212+ 0.02437 and 0.3068+ 0.01323 respectively. From aforementioned results, it is clear that, the estimated aflatoxin residues in samples were near similar to **Bohm et al., 2010)**. While **Sharma and Marquez (2001)** in Mexico recorded a lower result in 5.7%. **Martin sand Bernardo (2003), Boermans and**

Leung (2007). Failed to detect aflatoxin in dry pet food (dog and cat) samples. The results were attributed to generally very effective aflatoxin control within the European countries. In contrast **Fadeyemi and Akinrinde (2012)** the results show that, the range of concentration of total aflatoxins was 7.76 to 11.93 $\mu\text{g} / \text{kg}$ (mean: 9.61 $\mu\text{g} / \text{kg}$) in dry dog food. Dry food particles are usually formed through a process called extrusion, other methods include baking, flaking, pelleting, and crumbling of food to achieve a dry form, which protected against spoilage due to their low water content. The extruder uses a combination of steam, pressure, and temperature to rapidly cook foods. The extrusion process puts the ingredients through a temperature between 100 to 200°C and 34 to 37 atm pressures, which is high enough to effectively achieve a food sterilization process that meets industry standards. The resultant extruded material has moisture of approximately 25% before drying, where the final moisture content of 8 to 10% is attained. At this level of moisture mold formation is inhibited **Zicker, (2008)**; **Crane et al., (2000)**; **Millerand Cullor, (2000)**. In this study, author failed to detect total Aflatoxin in all examined canned pet food samples (Table 1). This is due to the cans are sterilized in a machine called a retort where temperatures of 121°C are maintained for a minimum of 3 minutes **Zicker, (2008)**. Moist or canned foods historically comprised a much greater segment of the manufactured pet foods market but they have decreased in use. Moist foods are high in water content, usually 60 to 87%, and require the presence of gelling agents such as starch or gums to achieve their final consistency. Moist foods go through a process that results in a well sterilized final product similar to canned products for human consumption. Ingredients are mixed, ground together, and then cooked into a hot mixture for transfer to the can. The slurry is allotted into the cans and the top is sealed under steam, which displaces any air, resulting in an anaerobic environment. **Zicker, (2008)**. Commercially prepared pet foods are an easy and economical way to fulfill the nutrient requirements in pets. These types of foods provide more than 90% of the calories consumed by pets in North America, Japan, Northern Europe, Australia, and New Zealand. Dogs, cats, hamsters, rabbits, birds, chinchillas and fishes are the main focus to pet food industry. Most manufactured pet foods are formulated to meet specific nutrient goals to support growth, maintenance, or gestation/ lactation as recommended by the **Association of American Feed Control Officials AAFCO, (2007)**. The nutrients that are targeted include the calories, protein, fat, carbohydrate, vitamins, and minerals required to sustain life and where possible, optimize performance **Zicker, (2008)**. Sorghum, maize, soya, rice, cereals, meal of meat and bones, by

products of birds, fish, chicken, derived product of egg and milk were the main ingredients of pet food **Sharma and Marquez, (2001)**. 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 **Puschner, (2002); IARC, (1993)**. 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. In terms of exposure and severity of chronic diseases (especially cancer), the mycotoxins pose a higher risk than anthropogenic pollutants, pesticides and food additives). coagulase-positive *Staphylococcus* (CPS). Of the 108 samples analyzed at only one sample; CPS **Galvão et al. (2014)**. Staphylococcal food poisoning is one of the most common foodborne illnesses and results from growth of certain strains of staphylococci in poorly handled or stored foods. In this study, author failed to detect Staph. Enterotoxins in all examined samples. If staph get into food and the food is kept at improper temperatures, the bacteria can grow. If the strain of staph that's in the food is one that can produce enterotoxins, these toxins can accumulate in the food at high enough levels to cause food poisoning when eaten. In most cases, people are probably the origin of enterotoxin-producing staph that contaminates food, but pets are another possible source.

Conclusion and Recommendation:

The results of this study provide information about total aflatoxin residues and staphylococcal enterotoxins in commercial dry food of dog and cat marketed in Egypt and reveals the level of mycotoxin in analyzed samples which were under the permissible limit.

Mycotoxin in decontamination in later stages of food production is difficult: it increases the cost of production and results are not always satisfactory **Paraica et al. (2002)**.

According to **Scott (1998)**. The decontamination process must.

- Destroy, inactivate or remove the mycotoxin;
- Must not produce or leave toxic, carcinogenic or mutagenic residues in final products or in food products obtained from animal fed decontaminated feed.
- Retain the nutritive value and acceptability of the products.
- Must not significantly alter important technological properties.

-Destroy fungal spores and mycelia which could, under favorable conditions, proliferate and for new toxins.

-More research is needed to better address the pet mycotoxin problem. Safety and efficacy of foods intended for animals are of prime interest to manufacturers because the health problems of pets are of a highly emotional concern, besides the pet food safety is the responsibility of the pet food industry. In the other hand, pet owners must care to store Thea nimal's food at home with regard to avoid fungal contamination, putting the open bags in aclean and dry place, with aeration and protected against humidity from environment. The shelf-life of commercial products must be observed, even at home.

-It is known that mycotoxin contamination in pet food poses a serious health threat to pets and recent problems with contamination, while affecting only a small percentage of commercial pet foods, impacted the entire pet food industry, affecting the confidence of veterinarians and owners. Long-lived, healthy consumers (pets) contribute to greater sales, so breakdowns in product quality can have catastrophic effect on profits or even company viability. More research is needed to better address the pet mycotoxin problem. Safety and efficacy of foods intended for animals are of prime interest to manufacturers because the health problems of pets are of a highly emotional concern, besides the pet food safety is the responsibility of the pet food industry. In the other hand, pet owners must care to store the animal's food at home with regard to avoid fungal contamination, putting the open bags in a clean and dry place, with aeration and protected against humidity from environment. The shelf-life of commercial products must be observed, even at home.

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دراسات غلي تواجـد بعض السموم الميكروبيـه في اغـذيه الحيوانـات الاليـفه

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قسم الفطريات (فرع شبين الكوم) و قسم صـحه الاغـزيه - معـهد بحـوث صـحه الحيوان - الدقي

الملخص العربي

تم فحص عدد 100 عينه من اغـذيه الكلاب و القطط (25 جافه و 25 معلبه) لمدي لتواجـد بقايا سموم الافلاتوكسينات الكليه باستخدام تقنيه الاليزا و ايضـا سموم المكروب العنقودي الذهبـي باستخدام Set-RPLA و قد دلت النتائج علي ان متوسط الافلاتوكسينات 0.02437 ± 0.3212 & 0.01323 ± 0.3068 في اغـذيه الكلاب و القطط الجافه , و لم يستدل عليها في اغـذيه الكلاب و القطط المعلبه كما لم يستدل عل سموم الميكروب المكور العنقودي الذهبـي في اي من العينات المفحوصه و تمت مقارنه النتائج بالحدود المسموح بها من قبل منظمه صـحه الدواء و الغذاء و تمت مناقشه الـاهميه الصـحيه لمثل هذه السموم عند تواجدها في اغـذيه الكلاب و القطط و اقترح التدابير اللازمه للتحكم فيها