**THE USE OF SOME NATURAL ADSORBENTS TO MINIMIZE THE ENVIRONMENTA POLLUTANTS IN POULTARY RATIONS**

**By**

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**The Researcher**

# Dedication

***

To the spirits of my mother and father,
may God bless their souls and endow them
in heaven.

To my devoted wife and my true-hearted
son, may God bless them both.

I dedicate this research to you, out of
recognition and gratitude.

The Researcher

# ABSTRACT

Mycotoxins are secondary metabolic products from moulds belonging in particular to the Aspergillas, Penicillium and Fusarium genera. More than 300 secondary metabolites have been identified although only around 30 have true toxic properties which are of some concern. Toxicogenic moulds may develop under all climatic conditions on any solid or liquid supports as soon as nutritional substances and moisture are present, hence the wide variety of contaminated foodstuff substrates. These toxins are found as natural contaminants in many feedstuffs of plant origin, especially cereals but also fruits, hazelnuts, almonds, seeds, fodder and foods consisting of, or manufactured from, these products and intended for human or animal consumption. Mycotoxins are small and quite stable molecules which are extremely difficult to remove or eradicate, and which enter the feed chain while keeping their toxic properties.

The present study was carried out as two experiments. Bentonite deposits were found reported in different parts of Egypt. In the present study, bentonite clay was selected as a local, cheap and readily available adsorbent for the removal of MB from the aqueous solutions. Natural and acid activated bentonites were characterized using XRD, FTIR and SEM. Adsorption of the dye was studied by batch adsorption experiments.Natural bentonite used is of montmorillonite nature as confirmed by the XRD analysis and chemical composition found in literature. FTIR and SEM analyses confirmed modification of bentonite treated with acid. This led to an increase adsorption capacity of activated bentonite. It was observed that the percentage of dye removal was improved from 75.8% for natural bentonite to reach 99.6% for acid treated bentonite.Results revealed that the adsorption of the dye increases with increasing the pH using natural bentonite. However, there was no effect of solution pH on the removal percentage of MB dye by acid activated bentonite. In addition, they indicated a gradual increase in the percentage removal of MB dye with temperature for natural bentonite. However, no effect was observed when acid activated bentonite was used. An optimum dosage of both natural and acid activated bentonite is $10g\ L^{1}$.

The adsorption kinetic studies showed that the removal of MB is a rapid process and the adsorption process obeys the pseudo-second order reaction, indicating cationic dye has a very strong affinity on the bentonite surface. A total number of 210 unsexed one-day Hubbard broiler chickens were randomly distributed into 7 treatments. The first group of chicks received the control basal diets. To be positive control , the second group of chicks was received contaminated control diet with aflatoxin to be negative control chicks of treatments 3, 4, 5, 6 and 7 received the contaminated control diets with Aflatoxins supplemented with 2% activated charcoal, activate bentonite, kaolin, natural bentonite and silicate, respectively. to investigate the effects of using some inorganic material as mycotoxins adsorbent on in aflatoxins contaminated diets on the productive performance carcass characteristics and histopathological examination of broilers chicks.

# INTRODUCTION

Feeds are exposed **1**to a plenty of environmental contaminants, which affects poultry that eat them then transferred to human when using their products including meat, and, eggs...etc.

Some examples of these contaminants are the residual of fertilizers, pests, and heavy metals, which source can be water, plant, or air. But the most dangerous of these contaminants at all recently are the poisons of Aflatoxins. They are produced from biological pollution, which results from the growth of fungi on feeds that may be found in spite of taking all safety measures in consideration that raw materials plunge in the structure of feeds from other different contaminants. This is because a lot of different environmental factors intervene in the growth of fungi resulted from biological pollution, and then excretion of fungal toxins. These factors are impossible to be controlled; such as temperature, humidity, ventilation, and light as well as the quality and structure of the substances intervened in the structure of the feeds, and the method and the duration of storing. Most of these are imported from abroad and are exposed to remain for long periods in transportation, storage, and humidity before manufacturing and this cause growth of microorganisms on them. Vaporization is followed to get rid of insects and fungi that grow on the raw materials intervene in the structure of the feeds. However, this procedure is not enough to eliminate the danger of such toxins because it eliminates only living fungi. Nevertheless their toxic excretions remained in feeds, and their toxic effect factors of vaporization and sterilization. Mycotoxins may reduce appetite and general performance. The adverse effects of mycotoxins on animal health is expressed in a diverse range of symptoms including haemostasis blood system damage skin lesions, immunosuppression, hepatotoxicity, nephrotoxicity, neurotoxicity, and even death .Avoidance of contaminated feeds is rarely feasible and feeds that contain relatively low concentration of aflatoxins may still have deleterious effects on sensitive species such as poultry **(Doerr et al., 1983).**

Such study aims at:

1- Reducing the ratio of the contamination of the aflatoxins by using the natural substances of adsorption, including the Bentonite, within the compounds of the feed.
2- Increasing the ability of the Bentonite for adsorption of toxins by its acidic activation for it.
3- Reducing the negative impact resulted from contaminating the feeds with Aflatoxins concerning the productive process to raise the poultry which in clades growth performances, weigh, the average of converting the feeds, carcass traits and histopathological changes of broiler chickens .

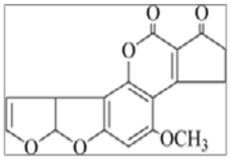
## 2 - REVIEW OF LITERATURE

**Aflatoxins** are a group of chemically diverse fungal metabolites that may pose health risks to animals and humans when consumed through feeding on defferants and food products. More than 100 mycotoxins have been structurally characterized, but few have been implicated in serious toxic syndromes. One of these few, aflatoxin, a group of highly carcinogenic, hepatotoxic, teratogenic, and mutagenic secondary metabolites of the fungi *Aspergillus flavus* and *Aspergillus parasiticus* that infect many crops have caused lethal episodic outbreaks of poisoning ***(Busby and Wogan,1984).*** Methods to limit infection of crops or to treat aflatoxin contaminated foods and feeds are of great economic importance to agricultureal production.The discovery of aflatoxin was triggered by the “Turkey X disease”, which struck Great Britain in 1960, and led to the loss of near 100,000 turkey pout’s from liver damage after they consumed aflatoxin-contaminated groundnut meal ***(Cullen and Newberne, 1994; Pereira Schuler, 2001).*** The scale of the loss and the serious economic damages caused by this incident stimulated intensive research***.(Sargeant et al. 1991)*** provided evidence that the mold *Aspergillus flavus* produced the toxic substances that lead to the disease in the turkeys (the name aflatoxin is derived from *Aspergillus flavus*). Similar results were soon discovered by researchers in other countries, and it was realized that many previous, less well-documented losses of turkey, ducklings, chickens, pigs, and other animals ***(Schoental,2001)*** which could be attributed to aflatoxin poisoning. A review of pre-1960 incidences is given by Eaton and Groopmanns , 1994 .The first aflatoxins to be recognized as the causative agent for toxicities were the aflatoxins B1, B2, G1, and G2,

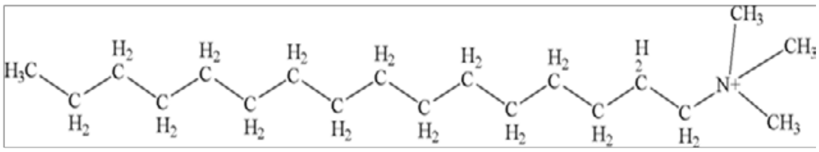
***and were characterized independently by (Van der Zidjen et al., 1990) and (De Iongh et al .,1962). (Asao et al., 1963)*** then elucidated their chemical structures.

## 2.1 Physical and Chemical Characteristics Mycotoxins

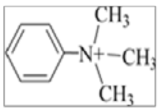
Structurally, mycotoxins are multi-heterocyclic compounds that contain a coumarone structure fused to a dihydrofurofuran (Figure 1) ***(Wogan, 1969)*** The AF analogues with an M notation have a hydroxyl group attached to the carbon between the furan rings***. (Grant, 1998).*** AFs are heat-stable and invisible to the naked eye ***(Phillips et al., 2002).*** They are intensely fluorescent in ultraviolet light. Of particular interest, AFB1 is a white crystalline solid with a melting point of 268-269 °C that emits a pungent smoke and irritating fumes upon decomposition. The compound has a molecular formula of $C_{17}H_{12}O_6$, a molecular weight of 312 g/mol, and is slightly soluble in aqueous solution where the peak absorbance in ultraviolet (UV) light occurs at 362 nm. Also, aflatoxines are soluble in methanol, acetone, and chloroform .but slightly soluble in hydrocarbon solvents ***(HSDB, 2003)*** Chemical characteristics of known AF metabolites are listed in **Table (1) and fig (1).** Additionally, AFs can be degraded by ultraviolet light ***(Phillips et al., 1994***



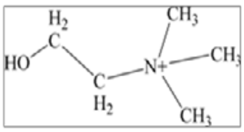
AfB1 - aflatoxin B1
fw = 312



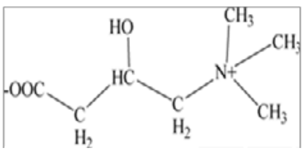
HDTMA - hexadecyltrimethylammonium
fw = 285



PTMA - phenyltrimethylammonium
fw = 136



Choline fw = 104
(essential nutrient in foods)



Carnitine fw = 161
(food supplement)

**Fig .( 1) :**

**Structure of some the Mycotoxins molecule.**

***(Wogan, 1969)***

| Aflatoxin | **formula** | **Molecular Weigh** | **Melting point** |
|---|---|---|---|
| $B_1$ | $C_{17}H_{12}O_6$ | 312.06 | 268-269 |
| [illegible] | [illegible] | [illegible] | [illegible] |
| $B_{2a}$ | $C_{17}H_{14}O_7$ | 330.08 | 240-DEC |
| $G_1$ | $C_{17}H_{14}O_7$ | 328.06 | 244-246 |
| $G_2$ | $C_{17}H_{14}O_7$ | 330.07 | 230 |
| $G_{2a}$ | $C_{17}H_{14}O_8$ | 346.07 | 190-DEC |
| $GM_1$ | $C_{17}H_{12}O_8$ | 344.28 | 276 |
| $GM_2$ | $C_{17}H_{14}O_8$ | 346.07 | 270-272 |
| $GM_{2a}$ | $C_{17}H_{14}O_9$ | 362.30 | 195-DEC |
| $H_1$ | $C_{17}H_{14}O_7$ | 330.07 | - |
| $M_1$ | $C_{17}H_{12}O_7$ | 328.06 | 299 |
| $M_2$ | $C_{17}H_{14}O_7$ | 330.07 | 293-DEC |
| $M_{2a}$ | $C_{17}H_{14}O_8$ | 346.08 | 240 |
| $MP_1$ | $C_{16}H_{10}O_7$ | 314.08 | - |
| $P_1$ | $C_{16}H_{10}O_6$ | 298.05 | >320 |
| $Q_1$ | $C_{17}H_{12}O_7$ | 328.06 | 295 |
| TI | $C_{17}H_{16}O_6$ | 314.08 | 234 |
| TII | $C_{17}H_{16}O_6$ | 314.08 | 233-DEC |
| 8.9-hydrodiol | $C_{17}H_{14}O$[illegible] | 346.07 | - |
| arasiticol | $C_{16}H_{14}O$ | 302.08 | 233-234 |

**TABLE( 1) : Chemical characteristics of aflatoxin metabolites**

***Heathcote and Hilbert (1978)***

## 2.2 Occurrence of Aflatoxins .

Aflatoxins are produced primarily by the fungi *Aspergilla's favas*, *A. parasiticus* ***( Lopez et al., 2002).*** Although members of this genus are distributed worldwide, the fungi are most abundant between latitudes 26° to 35° north or south of the equator ***(Klich et al.,1994).***

On the basis of these latitudes, it is evident that AFs occur mostly in tropical areas with hot and humid climates, prolonged drought stress, and insect infestation, all conditions that are favorable to the growth and development of molds

*A. flavus* and *A. parasiticus* reportedly have overlapping ecological niches and, under favorable pre-harvest conditions, they can produce AFs in developing seeds of corn, peanuts, cotton, almond, pistachio, and other tree nuts ***(CAST, 2003).*** However, the growth of the *Aspergillus* mold itself does not predicate the presence of the toxin, since production of AFs depends upon the complex interaction of many environmental and nutritional variables, including moisture, temperature, substrates, aeration,pH, the quantity of both carbon and nitrogen sources, lipids, certain metal salts, and specific nutrient requirements ***(Ominski et al., 1994 and CAST., 2003).***

For instance, the pronounced stimulatory effect of AF production has been linked to the level of zinc in host species, and has been cited as a contributing factor for the resistance of soybeans (with low levels of zinc) to AF contamination In addition, some of these factors may affect gene expression throughout the AF biosynthesis pathway ***(Bhatnagar et al, 2003).***

The process of AF contamination by *Aspergillus flavus* is best characterized in corn. *A. flavus* is a ubiquitous soil inhabitant that reproduces asexually by conidia (Payne, 1998). When the conidia are conveyed to corn silks by wind or insects, they can grow into the corn ear shortly after pollination and colonize the kernel surfaces ***(Widstrom, 1996)*** Under favorable environmental conditions, the fungus may directly penetrate the seeds and cobs or it may enter through wounds created by insects. In either scenario, significant AF production and contamination do not occur until the moisture content of the kernel is below 32%  and continues until the grain moisture reaches 15%.   The optimal temperature for the growth of these fungi ranges from 36 to 38 °C ***(Klich et al., 1994).***   In field studies, AF contamination  in peanuts by *A. favas*  were maximized at 30.5 °C ***(Cole et al., 2006).***

Recent studies estimated that mycotoxins contaminate 25% of the world's food crops and account for more than $1.4 billion in economic loss in the United States alone ***(Bingham et al., 2004).*** Due to warm humid climate in the Southern United States aflatoxin contamination is widespread. According to an article in the Wall Street Journal, drought stress triggered an outbreak of aflatoxin in the Midwest of the United States in October 2005 ***(Kilman, et al 2005).*** In Iowa, as much as 20% of the corn brought to an elevator after harvest was found to have worrisome levels of aflatoxin. Heavily contaminated feeds are usually destroyed or are sold at a steep discount for non-feed uses. In the 90s, farmers in Texas suffered several consecutive years of economic hardship from contaminated corn crops.

In farm animals, even low levels of mycotoxicosis induced by aflatoxins  are correlated with feed refusal, reduced feed conversion ratios, anaemia, reproductive failure, impaired immune response and renal damage Similar symptoms can be found in hatchery-reared fish. Higher doses of aflatoxin are often lethal. One of the numerous more recent examples is the contamination of dog food that repeatedly lead to the deaths of dogs in Texas as reported by***( Bingham et al., 2004).***

Aflatoxins are well recognized as a cause in liver cancer and toxic effects, not only in animals but in humans as well. In June 2004 the BBC reported in a news article on their website about an incidence in Kenya with more than 180 local people who had to be hospitalized due to the consumptions of aflatoxin with contaminated corn. They were suffering symptoms of liver failure, yellow eyes, vomiting and bleeding from their nose. Eighty of those people died from this aflatoxin poisoning. Directly correlating certain disease in humans, such as the occurrence of liver cancer, to the consumption of aflatoxin contaminated food as the causative agent is difficult. However, there are an estimated 4.5 billion persons living in developing countries who are chronically exposed to uncontrolled amounts of the ***toxin (Williams et al., 2004)*** give a thorough overview over different aspects of aflatoxicosis in humans in developing countries. For the United States***,(Wood ,1999)*** found "no direct evidence that implicated aflatoxins as the causal agents for human cancer***".( Stoloff ; 2006)*** published a probability study on aflatoxin as a cause for primary liver-cell cancer in men in the United States concluding that at the present time it is not possible to correlate the chronic carcinogenic symptoms in men as opposed to acute toxicities from high levels of aflatoxin such as mentioned above. However, correlations of aflatoxins with carcinogenetic have been found when tested in laboratory animals. Therefore, presence of aflatoxins should be restricted to the lowest practical level.

The US Food and Drug administration (FDA) considers aflatoxin to be an unavoidable food and feed contaminant, and it is the declared goal to minimize contamination by implementing regulations that require the survey and management of the problem. They set action levels that consider agricultural imported or domestic shipments adulterated at aflatoxin levels exceeding 20 ppb. The FDA action levels are listed in **Table ( 2).**

To reduce or solve the problem of aflatoxin contamination, there have been several different approaches ranging from physical separation of contaminated kernels to chemical treatment to degrade the toxin Management practices can prevent or minimize the degree of aflatoxin occurrence of the produce in the field or storage ***(Riley and Norred, 1999).*** Once contaminated, aflatoxin detoxification measures of post-harvest treatment to remove or reduce the toxic effects need to be undertaken.

Possible strategies to destroy aflatoxin during food and feed processing are discussed by ***(Phillips ,1994)*** and include thermal inactivation, irradiation, solvent extraction and mechanical separation, density segregation, bio-control and microbial inactivation, ammoniation, treatment with bisulfide, heterogeneous catalytic degradation and several other chemical treatments. Despite improved handling, processing and storage, aflatoxin remains a problem in the food and feed producing industries. Besides degradation of Aflatoxins by ozonation (Proctor,