



## EFFECT OF AFLATOXINS ON POULTRY PRODUCTION AND CONTROL METHODS OF DESTRUCTIVE INFLUENCE

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### ABSTRACT

Aflatoxins are natural fungi toxins that have originated from *Aspergillus* fungi and secondary metabolites of moulds such as *Aspergillus flavus* and *A. parasiticus*. These toxins can be carcinogenic if they enter the body through food. Aflatoxin toxins can cause tissue necrosis, hepatic cirrhosis and liver cancer. Observed clinical signs in human including: regurgitation, cramp, acute hepatic lesions such as lipid changes, pulmonary edema, muscle tremors, coma, hysteria and death with brain edema and involvement of organs such as liver, kidney and heart. Among aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, the most common ones is aflatoxin B<sub>1</sub> that is very toxic. Aflatoxins can be found in many foods, for example, grains, oilseeds, spices, maize, groundnuts(peanuts), pistachios, red pepper, black pepper, dried fruit, figs, are substances that have a high risk of aflatoxin. But this dangerous toxin has been observed in a wide variety of food. There has been a chance of contaminant with aflatoxin M in milk, cheeses and other dairy products; and Mycotoxins have had improper economical effects on farmers, consumers of agricultural and animal products and ultimately the entire of community.

**Keywords:** aflatoxin, poultry, immune, enzyme.

### INTRODUCTION

Food has an important role in people's life. Animal protein is one of the essential needs of human nutrition. Poultry has been used as a valuable source of protein in human nutrition in the world for years and animal products, specially meat and poultry liver have played an important role in people's nutrition. This product is influenced by factors that make it improper nutrition uses because of different reasons (Allameh and Razzaghi Abyaneh, 2001). Today, aflatoxin-polluted agricultural products are one of the most important community problems and different countries have legislated special laws for food production, consumption and importation (Allameh and Razzaghi, 1381). For example, in US, food and drugs with more than 20ppb total aflatoxin and 15ppb aflatoxin B<sub>1</sub> have not been suitable for buying, sales, imports and exports (Gourama and Bullerman, 1995). During second world-war, poultry production and consumption significantly increased when beef and pork supplies were limited. Storage improvement methods and chicken and egg dispensation have been

facilitated to this increased food consumption and meat and egg including as a meal animal protein supply (FAO, 2010).

Toxin-producing fungi are found everywhere in the environment and they can enter our food and produce toxic secondary metabolites called mycotoxins (Bagher Zadeh Kasmani, 2012). Humans and animals have affected various mycotoxins (Table-1) with different degrees of toxicity (Jelinek *et al.* 1989). Aflatoxin-caused health disorders have been one of the reasons for low life expectancy in developing countries (Williams, 2004). Since aflatoxin isolation and identification, these compounds have had a special place in human and animal health because of different biological effects (including carcinogenesis, mutagenesis, malformations, hepatotoxicity, renal and skin toxicities and efficacy of immunosuppressive) and different biochemical effects (including the effects of them on carbohydrates and lipid metabolism, protein and nucleic acid synthesis) (Dudhe *et al.* 1998).

**Table-1.** The major toxigenic species of fungi and their principal mycotoxin.

Fungal species	Mycotoxin
<i>Aspergillus flavus</i> ; <i>A. parasiticus</i>	Aflatoxins
<i>A. flavus</i>	Cyclopiazonic acid
<i>A. ochraceus</i> ; <i>Penicillium viridicatum</i> ; <i>P. cycloium</i>	Ochratoxin A
<i>P. expansum</i>	Patulin
<i>Fusarium culmorum</i> ; <i>F. graminearum</i> ; <i>F. sporotrichioides</i>	Deoxynivalenol
<i>F. sporotrichioides</i> ; <i>F. poae</i>	T-2 toxin
<i>F. sporotrichioides</i> ; <i>F. graminearum</i> ; <i>F. poae</i>	Diacetoxyscirpenol
<i>F. culmorum</i> ; <i>F. graminearum</i> ; <i>F. sporotrichioides</i>	Zearalenone
<i>F. moniliforme</i>	Fumonisin
<i>Acremonium coenophialum</i>	Ergopeptine alkaloids

The presence of mycotoxins may cause reduced feed intake, reduced feed efficiency, utilization rates and increased risk of infection and cause fertility problems (Marquardt, 1996). Generally, changes in some blood and serum biochemical indices of birds would be raised due to aflatoxicosis. Reported changes in blood indices including decreased hematocrite, hemoglobin, red blood cell counts and lymphocyte percentage and increased white blood cell count and hydropylo percentage (Raju and Devegowda, 2000). Various methods have been used in order to remove aflatoxin contamination of food and food sources. In all these methods including physical, chemical and biological ones, the main objects have been toxin dissociation, destroying, destruction and inactivating.

Using of additive materials, such as Selenium, have been suggested as for to impossibility of removing Aspergillous fungi and other toxin producing fungi from poultry food, and poultry has been the main consumption protein in our food basket. Selenium is kind of rare element has played an important role in animal life, especially vertebrates. In recent years, Selenium importance has been characterized in animal and poultry nutrition. Participation in Glutathione peroxides structure is one of the most important roles of this element. In other hand, it has been identified that when particle sizes reducing in nano-scale, new properties such as quantum effect and high reactivity have been revealed in a wide range (Zhang *et al.*, 2001). Selenium has different effects including anti-carcinogenic, detoxification, protection against oxidative damages or aging, animal protection against muscle nutrition disorder and as well as being nutrition for treating AIDS. Also this element has had exclusive properties in biochemistry and molecular biology, therefore, have make researching in nutritional science to an exciting goal (Hatfield, 2001). Peng *et al.*, (2014) have studied Sodium Selenite protective role

in aflatoxin B<sub>1</sub>-induced apoptosis in broiler jejunum and have concluded that 0.4 mg/kg Selenium Sodium supplement of broiler jejunum has protected from developmental delays, reduced proliferation and inhibition of aflatoxin-caused G2/M phase transition.

#### Mycotoxins and economical effects

Poor economical impacts of mycotoxins have been on farmers, agricultural and animal-product consumers and ultimately the entire of community. Grain producers and grain maintainers will be affected due to the reduction in farm operations, and cereal storage and testing costs, respectively. Also grain grower should be sustained high price with the loss of agricultural production. In the other hand, agricultural products consumers have suffered irreversible complications arising from the use of infected products, too. Finally, society as a final costumer must pay exorbitant costs of increased regulation and research, low exports and high imports and treatments. Currently, these costs are found at every level of grain production and estimating of accurate wasted cost is impossible (Umayya, 2011).

#### Chemical structure of aflatoxin

Chemically, aflatoxins, which are closely related in structure, form a high-oxygenated heterocyclic compound (complex furanocoumarins). Aflatoxins have a B-fused coumarin nucleus (Table 2), while aflatoxins B have acyclic cyclopentane form; M and G groups are results of B group hydroxylation and Kanton ring, respectively (Bullerman and Gourama, 1995). Aflatoxin B<sub>1</sub> with molecular weight 312 and the formula C<sub>17</sub>H<sub>12</sub>O<sub>6</sub> has shown relatively strong blue fluorescence against UV. This aflatoxin in colorless crystals formation has been disintegrated at temperatures of 268-269°C melting points (Goldblatt, 1997; James, 1997).

**Table-2.** Physical and chemical important properties of the Aflatoxin.

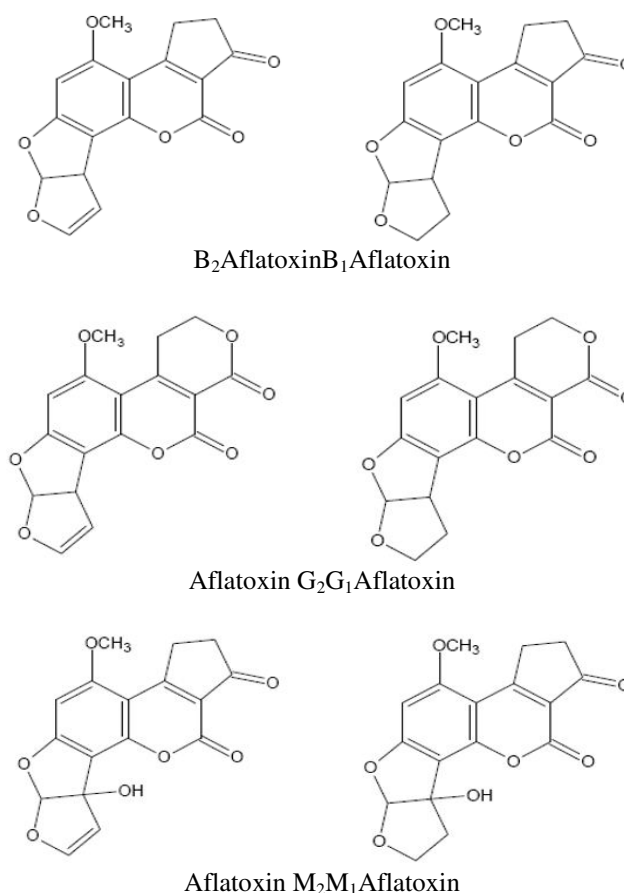
Aflatoxin	Molecular formula	Molecular weight	Melting point	UV absorption max UV( $\epsilon$ (L mol <sup>-1</sup> cm <sup>-1</sup> )), metanol	
				265 nm	360-362 nm
B <sub>1</sub>	C <sub>17</sub> H <sub>12</sub> O <sub>6</sub>	312	268-269	12400	21800
B <sub>2</sub>	C <sub>17</sub> H <sub>14</sub> O <sub>6</sub>	314	286-289	12100	24000
G <sub>1</sub>	C <sub>17</sub> H <sub>12</sub> O <sub>7</sub>	328	244-246	9600	17700
G <sub>2</sub>	C <sub>17</sub> H <sub>14</sub> O <sub>7</sub>	330	237-240	8200	17100

### Factors affecting Aspergillous growth and toxin production

Fungi need 24 to 35°C temperate and humidity over 7% for growing and aflatoxin production (Williams *et al.*, 2004). Aflatoxin production value is different based on products, soil and dryness condition which caused damage to products (Brown *et al.*, 2001; Bankole and Mabekoje, 2004). Heavy rain during harvest has resulted in increased product humidity, and also damage of protective shell by insects has helped to fungi colonization and toxin production before harvesting and during storage. Food storage without suitable condition and humidity has caused absorption of Aspergillous infection and aflatoxin production (Hell *et al.*, 2000; Ono *et al.*, 2002; Hawkins *et al.*, 2005; Turner *et al.*, 2005).

### Aflatoxin interaction with DNA

Aflatoxins (Figure-1) can bind to DNA and change its molecular structure. Aflatoxin B<sub>1</sub>, biologically the most active aflatoxin, reacts stronger than aflatoxins G<sub>1</sub> and G<sub>2</sub>. It has been identified that the main difference between aflatoxins and other DNA-reacting compounds base on aflatoxin interaction with Purina and its derivatives which is followed-up by spectroscopy, is that aflatoxins can also interact with single-string DNA. This interaction can be explanation of simultaneously prevention of DNA and RNA synthesis by aflatoxin. Nonetheless, aflatoxin binding with DNA is so weak that simply passing through a chromatography column has separated this connection (Alleroft, 1964; Goldblatt, 1997).

**Figure-1.** Chemical structure of Aflatoxins.

Aflatoxin, especially Aflatoxin B<sub>1</sub>, is widely toxic for gene and this has caused DNA damage, gene mutation, chromosomal malformations and in vitro mammalian cell to cell transition.

### Toxic effects on chickens

When the chicken is fed with aflatoxin-contaminated feed, the liver, kidneys, immune system and thus the performance of birds will be affected. Aflatoxin toxicity is related to biochemistry, hematology, reproduction and poultry pathological changes (Ortatatli AND Oguz, 2001)

Moreover, Aflatoxin residues in meat and eggs would pose a threat to the health of consumers. Most of the times, acute aflatoxin have caused mortality of birds. On the other hand, chronic aflatoxin exposure has not been



significant in the field but acute doses may affect bird biochemical situation and cause reduced overall performance. Therewith, the rises of chronic or acute toxicity of broilers have depended on duration of exposure and body metabolism (Kermanshahi *et al.*, 2009; Shabani *et al.*, 2010). Approximately, aflatoxins have endangered all poultry production parameters. In this relation, weight has affected nutrition, feed conversion coefficient, and egg production, male and female reproduction. Deleterious and desirable effects of aflatoxin on poultry performance depend on toxin dose and duration of exposure to the toxin. The main clinical signs in meat cattle with aflatoxicosis have included delayed development and decreased body weight. Other symptoms can be the amount of food and water, neurological symptoms such as weakness of the legs and wing failure and finally death (Reddy *et al.* 1989). Other effects of aflatoxin toxicity have related to act on phagocyte cells, the circulatory system and reduced resistance to infectious agents. Weakened immune system due to aflatoxin toxicity has made birds susceptible to some infectious diseases such as coccidiosis, infectious bursa disease, and respiratory infections (Gupta *et al.* 2003).

Fani Makki *et al.* (1329) have studied different levels of aflatoxin B<sub>1</sub> on intestinal length, blood parameters and immune system in broiler chickens. This study no significant changes in calcium, phosphorus, albumin and total protein in the serum of experimental of broilers compared with birds receiving the basal diet. As the levels of Aflatoxin B<sub>1</sub> from 250ppb to 500ppb in poultry feed, the serum levels of glucose, total bilirubin and glutathione peroxidase also increased. In contrast, the level direct bilirubin decreased significantly. HI titers against influenza and Newcastle diseases in the second stage (34-days) showed a significant decrease compared to the basic treatment. Aflatoxin has caused changes in blood parameters, kidney and liver damages and decreased immune functions against Newcastle disease and influenza in broiler chickens.

Saiedi (1384) has compared the effect of aflatoxin B<sub>1</sub> as a carcinogenic agent in activity and development of hepatic metabolized enzymes and the binding level of aflatoxin B<sub>1</sub> to DNA in immature rats with adults. In this research, the activities of glutathione peroxidase and microsomal cytochrome P450 with their changes in different post-birth ages were measured by using the absorption method which has indicated that the activity of aflatoxin metabolized enzymes in prepubertal ages was less than puberty one and a significant difference was observed. Immature rat liver has had less capacity in metabolism of toxic and strange carcinogenic agents such as aflatoxin B<sub>1</sub> and it has caused excretion of these substances and storage of them in their body that might permanently exposed them to carcinogenic agents and hepatic damage finally.

### Strategies to control and eliminate aflatoxins

Pre-harvest strategies such as maintenance of the plant and good growth condition, enough fighting against anti-fungi and insects and weed prevention can be

mentioned to prevention of aflatoxin infection. Using appropriate harvest equipment, clean and dry transportation equipment and also suitable harvest conditions have been recommended during harvest. Moreover, post-harvest strategies such as drying of harvested grain, proper storage conditions and using optimal and free-fungi growth transport vehicles have been recommended, too (Park, 2002).

### Methods to reduce toxicity

Lots of antiseptic methods have been created to wash out food toxicant and reducing infectious-production toxic signs. These are biological, physical and chemical methods.

### Physical methods

Screening and grading of aflatoxin-contaminated grain in peanuts has been suggested as an effective method. Aflatoxin concentration should be reduced by food drying for two days. Also, drying food with dry heat in 80 to 100°C temperature for 6 to 8 hours has been an effective method to reduce aflatoxin of food. In addition, food drying under the sunlight has caused loss of side effects in the sheep (Gowda *et al.* 2009). Moreover, the use of surface absorbent such as bentonite and hydrated sodium calcium aluminosilicate in contaminated feed has proven to be effective in reducing the bioavailability of aflatoxin in animals. It has been shown that Calcium Montmorillonite is a safety absorbent for humans (Wang *et al.* 2005). Microorganism potency such as yeast and bacterium has been examined by using this method in changing or inactivating aflatoxin of human-using products (Zaghini *et al.* 2005).

### Chemical methods

Chemically, aflatoxins can be washed out with calcium hydroxide, mono methyl amine, ammonia and ozone. Among all chemicals, massive ammonia was used for cottonseed meal, peanut meal and sunflower. But the main forms of using chemical substances have fallen back to the risk of animal health (Galvano *et al.* 2001).

### Biological methods

*Flavobacterium urantiatum* can delete aflatoxin B<sub>1</sub> from liquid medium and its application is in the peanut production as biological parser. In recent years, lactic acid bacteria have been studied as an in vitro-field aflatoxin liquidator (Diarra *et al.* 2005).

### Antioxidant usage

In recent years, there has been an increased interest among poultry scientists in using antioxidants against aflatoxin toxicity. This is because aflatoxins have helped to induce the production of reactive oxygen and oxidative stress and have been suggested as one of the underlying mechanisms of cellular damage and DNA damage (Yang *et al.* 2000). Free radicals produce during natural cell metabolism. Free radicals are molecules or molecular fragments that contain one or more unpaired electrons in their atomic or molecular orbital. There are



different kinds of free radicals but only some of them that are derived from oxygen and related to active oxygen are important in biological systems. Continuous reduction of oxygen molecules has led to the formation of a group of reactive oxygen species such as superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), hydroxyl radical (OH) and oxygen. Unpaired electron of free radicals has a great affinity with neighboring molecules such as lipids, proteins and carbohydrates and has been suggested as the major cause of cell damage (Halliwell, 2000). Aflatoxin is a small molecule that is rapidly absorbed in the digestive tract by passive diffusion and transmitted to liver. In the liver, aflatoxin B<sub>1</sub> has been metabolized by cytochrome P450 enzyme and become in the form of aflatoxin B<sub>1</sub>-Exo8-9-Epoxides (AFBO). This Epoxide has linked to the guanine N7 position and become in form of adduct compound AFB-DNA, a compound that mainly responsible for the carcinogenic and mutagenesis effect of aflatoxin. However, no carcinogenic effects have been recorded in poultry but it can bind to cell modifier molecules. In the second stage, aflatoxin, specially AFB<sub>1</sub>, produce reactive oxygen species such as Superoxide anion radicals, hydrogen peroxide and lipid hydroperoxides which are precursor of hydroxyl radical.

These hydroxyl radicals have an affinity with DNA and affinity to produce mutations (Ozen *et al.* 2009). In addition to increasing lipid peroxidation, it has been confirmed that aflatoxin has reduced activity of antioxidant enzymes in bird tissues with aflatoxicosis in the field condition (Umayya & Parvatham, 2009).

Previous studies have been focused on aflatoxin function, biochemical changes and pathology but with the advent of molecular biology, investigations have uncovered molecular changes caused by aflatoxin in broilers and useful information has been acquired for researchers and poultry industry. Aflatoxin has induced changes in gene expression patterns. Feeding 2 ppm aflatoxin to chickens for 21 days will be effected in the most up-regulation of genes related to biotransfer and cell proliferation and disrupting the genes involved in immune, antioxidant function and oxidative phosphorylation.

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