PREVENTION AND CONTROL OF MYCOTOXINS IN FEEDS

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REVIEW ARTICLE

ABSTRACT:

Mycotoxins are compounds produced by toxigenic strains of fungi in grains and feedstuffs. Mycotoxins can contaminate agricultural products before harvest, after harvest, during processing, transport and storage. The contamination levels are influenced by environmental factors and insect damage.

Most of the mycotoxins are of greatest potential risk to humans and animals. The toxicity of mycotoxins to animals range from acute death to chronic disease and interference with reproductive efficiency. Contamination of meat, milk and eggs can result from animal consumption of mycotoxin contaminated feed or feed-ingredient. These contaminants of mycotoxins and their metabolites are hazardous to humans.

Management practices to avoid mycotoxin accumulation in stored grains or feed are conducted by several authors. Optimization of environmental conditions, good sanitation practices, addition of antifungal agent of low toxicity to animals and good insect/rodent control are valuable. Moreover, several procedures for the decontamination/detoxification of mycotoxin containing feeds have been reported. These include physical separation, thermal inactivation, irradiation, microbial degradation and chemical treatment. This article summerises the different methods used for prevention and control of mycotoxins in animal feeds.

METHODS OF PREVENTION AND CONTROL:

Prevention of the contamination of fungi and their mycotoxins in agricultural commodities or feed ingredients can be divided into the following three levels:

I-PRIMARY PREVENTION:

Several hundreds (probably thousands) of fungus species have the capacity to produce toxins. All these fungi have three critical environmental requirements (temperatures above freezing, moisture above 14% and oxygen). Limiting any one of these requirements will reduce or prevent the production of toxins (Gotlieb, 1999).
Primary prevention should be initially carried out before the fungal infection and mycotoxin production. Several practices have been recommended to keep the conditions unfavourable for any fungal growth (Suttajit, 2002). These include:

1-Control of the environmental and other factors that influence fungi invading:

A-Pre-harvest control:

Pre-harvest control is the first step in ensuring a safe product. Once the crop becomes infected under field conditions growth will continue during post-harvest stages and storage (Lopez-Garcia et al., 1999). The pre-harvest management is focused on controlling critical factors that have been shown to enhance mycotoxin production, such as:

Irrigation and soil condition: High moisture and high relative humidity are essential for spore germination and fungal proliferation. Therefore, adequate efforts should be made to avoid extreme conditions of either drought or excessive moisture (Lopez-Garcia et al., 1999).

Weed control: A sound weed control is necessary for optimum yield and crop quality but also help in reducing level of inoculum (Janet and Canale, 1997).

Control of insect infestation: Control of insect infestation in the field may help to prevent proliferation and subsequent mycotoxin production (Suttajit, 2002).

Management of crop residues: When the crop is harvested, some residues remain in the field, these provide an environment that is conducive to the survival of fungal spores and the subsequent infection of the next crop. Proper management of crop residues would help to avoid this problem (Lopez-Garcia et al., 1999).

B-Post-harvest control:

Post-harvest control and decontamination procedures represent an important tool in avoiding consumer exposure to mycotoxin.

Dry the harvest grain: Grain and oil seed crops should be harvested at their optimum maturity and dried as soon as possible to moisture content levels that not allow fungal growth (less than 14%) (Jones et al., 1994; CAST, 1989). Sun drying is probably the oldest and most common way of reducing the moisture content of any crop. It has been practiced while the crops are still in the field (Noomhorm and Cardoma, 2002). Other methods of grain drying include mechanical drying, in-bin drying, infrared, microwave or sonic and solar energy drying (Goyal, 2002).

Control the physical condition of grain: Damaged portions of crops should be removed during the harvesting process whether the damage is caused by physical or biological causes. Care should be taken to remove foreign materials and high moisture plant parts during harvesting (CAST, 1989).

C-Good management of feed bins (stores):

Good management of stores will minimize mold related problems. A good management programme should include the following points:

-Chick bins for leaks and poor ventilation (Vest, 1997). Bins Should have good ventilation to minimize heat buildup and sweating of side walls (Waldroup, 1997).

-Minimize feed storage time and do not leave feed in bins between flocks (Vest, 1997). Ground feed is an ideal source of food for fungal growth, therefore it should be utilized rapidly (Royes and Yanong, 2002).

-Clean storage bins regularly, particularly roof areas and upper inside walls (Vest, 1997), and remove old grains or feeds, damaged kernels.
and any foreign matter from bottom of the bin (Boyles and Eastridge, 2002), to prevent bridging of the feedstuffs and creation of hotspots (Royes and Yanong, 2002).

Bagged feed: Storage of finished feed or raw material in bags presents problems especially, in humid climates. Condensation and mold growth can occur easily in solid walled and lined bags as temperature change. Jute and plastic mesh bags are particularly troublesome if recycled and used more than once for feed. Furthermore, the open nature of these bags allows humidity and mold spores to penetrate to the feed within (Waldroup, 1997).

-Grains with elevated moisture should be aerated and/or dried with heat (Boyles and Eastridge, 2002).
-Chick grains periodically for temperature, moisture and insect damage (Boyles and Eastridge, 2002).

Grain temperature, moisture and duration interact in regulating the growth of storage fungi. Table (1) lists safe storage duration for corn, depending on grain temperature and moisture. Note that from a starting point of 14% moisture and 50°F, every 10°F increase in temperature or 1.5% increase in storage moisture reduces the safe storage period by half (Raney et al., 1987).

Table (1): Safe storage duration for corn held at specific moistures and temperatures.

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<tr>
<th>Temperature (F)</th>
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N.B. When considering silage, it is neither practical nor desirable to limit temperature or moisture. Limiting oxygen is the key to successfully limiting toxin production during ensilement. Oxygen is like a light switch. It can turn toxin production on and off during storage (Gotlieb, 1999).

Silage: Discard mouldy areas when silo is first opened or when moldy pockets are found during feeding, and feed sufficient rate per day to minimize spoilage (Boyles and Eastridge, 2002).

d-Control infestation in stored bulk grains:

Insects damage grains by removing the germ, eating the endosperm and cotyledons, exposing external layers of the seed to the dust, depositing fecal pellets, dying and leaving their mortal remains in the grain, and creating favorable conditions for the development of fungi (Williams, 1991) and can also serve as carriers of fungal spores (Harris and Staples, 2002). Once fungal growth starts, the water of
metabolism from the fungus will provide sufficient water for further growth and mycotoxin development (Maracas and Nelson, 1987).

2-Development of fungal resistant varieties of growing plants:

It has been clear that the fungal-resistance of each variety is genotypic. For example the resistance to invasion of A. flavus has been attributed to several biochemical, environmental and physical factors. Some strains would require physical damage for their infestation and others would not. During long grain storage, the biochemical activity of grain is much reduced, while invasion of storage fungi and mycotoxin contamination would increase (Suttajit, 2002).

Genotypes of peanuts and biochemical properties of its seed such as tannin content, thin pericarp (Rao and Tupule, 1967), small amount of circular wax (La Prade et al., 1973), and chemical composition of the pericarps and embryos (Lindsey and Turner, 1975) have been shown to inhibit fungal invasion by A. flavus and aflatoxin formation.

Recently, antifungal enzymes, chitinase (Roberts and Selitrennikoff, 1986) and B-(1-3) D-glucanase (Nelson et al., 1969), found in a number of plant seeds, may acts as defense against pathogenic fungi, since chitin and glucan are major polymeric components of many fungal cell walls. Such polysaccharides in fungal cell wall could be enzymatically hydrolysed into smaller products resulting the damage or killing of fungal mycelia or spores. It is foreseen that seeds rich in such antifungal enzymes likely resist the infestation of fungi. Even there are many technical problems in searching for the "super" plant against pathogencity, the development of fungal-resistant plant verities utilizing genetic resistance to mycotoxin contamination is still possible and encouraged (Suttajit, 2002).

On the other hand, studies have identified the chromosome regions associated with aflatoxin resistance. This line of research is therefore a good option for future pre-harvest control and prevention of mycotoxin formation. Genetic engineering has also been useful in the development of host resistance through the addition or enhancement of antifungal genes. Many endogenous compounds with low molecular weight and bio-macromolecules in maize kernel tissues have been identified as antifungal compounds. Enhancing the production of the compounds may also enhance resistance to mycotoxin contamination (Lopez-Garcia et al., 1999).

3-Use antifungal agents (mold inhibitors):

Antifungal agents used to prevent fungal growth have no effect on the toxin already formed. The main types of mold inhibitors are:

-Organic acids e.g. acetic, propionic, butyric, benzoic, formic, lactic and sorbic acids (Sherwood and Puberdy, 1974; Suttajit, 2002).

-Salts of organic acids e.g. sodium or calcium propionate and potassium sorbate. Generally the acid form as a mold inhibitor is more effective than its corresponding salt (Jones et al., 1994). The critical concentrations of all these acids, whether used singly or in combination was found to be between 0.1 and 1% W/W. Below 0.1% the fungus was able to develop (Sherwood and Peberdy, 1974). According to Herting and Drury (1974) propionic and formic acids are effective antifungal agents or corn, grain sorghum, wheat, oats, barley and soybeans. Propionic acid was found by Sauer and Burroughs (1974) to be effective as a mold inhibitor for corn and
grain sorghum at moisture contents of 18 to 24%, isobutyric, acetic and formic acids followed, in order. The particular value of propionic acid as a preservative stems from its low order of toxicity towards human and other animals. In fact, it is a normal component of the digestive tract of ruminants (Kiessling and Pettersson, 1991). A positive effect of this was observed by Jones et al. (1970) when they reported that propionic acid, besides being an effective preservative of high-moisture corn, also brought about an improvement in animal performance. Grains treated with propionic acid can be used only for livestock and poultry feeding (Jacobsen et al., 1993). Mold inhibitors (sodium or calcium propionate or organic acids) are added to stored grains to prevent further development of molds at levels of 0.2 to 0.25% to feeds with 14-17% moisture and 0.5 to 0.6% to feeds with 18-24% moisture (Tarr, 1996).

- Benzoic acid derivatives e.g. O-nitrobenzoate, O-aminobenzoate, P-aminobenzoate, benzocaine (ethyaminobenzoate) ethyl benzoate, methyl benzoate and o-acetoxy benzoic acid (Davis and Diener, 1967).

- Potassium sulfite and potassium fluoride (Suttajit, 2002).

- Fumigants: Ammonia and phosphine (Vandergraft et al., 1975).

- Gentian violet (0.5-1.5 g/kg) and thiabendazol (100 mg/kg) (Luxsanakoses, 2002).

- Copper sulphate: Copper sulphate is a poor mold inhibitor for poultry feeds. Moreover, excessive levels of copper are toxic to young animals and sheep and will accumulate in the environment (Jones et al., 1994).

  There are many factors influencing the effectiveness of mold inhibitors (Jones et al., 1994), these include:

- Particle size: Mold inhibitors can not be effective unless they are completely and thoroughly distributed throughout the feed. The smaller the inhibitor particles, the greater the effectiveness.

- Feed ingredients: Protein or mineral supplements e.g. fish meal, poultry by-product meal, soybean meal and limestone tend to reduce the effectiveness of propionic acid. These materials can neutralize free acids and convert them to their corresponding salts, which are less active as inhibitors. Dietary fat tends to enhance the activity of organic acids probably by increasing their penetration into feed particles.

- Pelleting of feed: Pelleting feed has among its numerous benefits, destruction of fungal spores and a decrease in the fungal burden. Also, the heat produced during pelleting of feeds enhances the effectiveness of organic acids. Generally, the higher the pelleting temperature, the more effective the inhibitor.

- Organic acids are corrosive to metals and irritating to skin, but some have been modified to counteract this characteristic. Concrete or steel surfaces that will contact acid-treated feed should be covered with plastic or coated with acid resistant paint.

4. Animal management:

- Moldy or toxic feeds should be removed and replaced with unadulterated one.

- Acidic diets should be avoided as they intensify the effects of mycotoxin (Jones et al., 1994).

- Addition of nutrients such as proteins, amino acids (choline, methionine), energy (fats and carbohydrates), vitamins and trace elements to animal diet may also be advisable (Jones et al., 1994). Some of these have effective, while others appear to have little effect. Crude protein: Because aflatoxin metabolites appear to have an effect on protein and amino acid
metabolism, researchers have examined the possibility of increasing dietary protein levels as a means of alleviating aflatoxicosis (Waldroup, 1997). Smith et al. (1971) demonstrated that increasing the crude protein levels alleviated the growth depressing effects of aflatoxin. For ochratoxin A, Gibson et al. (1989) observed that increasing dietary crude protein helped to alleviate but did not eliminate the adverse effects of ochratoxin A (OA) on body weight and feed conversion, but mortality rates did not appear to be affected. However, increasing protein levels is a costly approach to mycotoxins control (Waldroup, 1997).

Methionine: Increasing the dietary total sulfur amino acids to levels in excess of NRC protected chicks from the growth depressing effect of aflatoxin, possibly through an increasing rate of detoxification by glutathione, a sulfer amino acid metabolite (Veltmann et al., 1981 & 1983).

Phenylalanine: In virtue studies have indicated that the toxicity of OA may be alleviated by phenylalanine supplementation (Creppy et al., 1980; Klinkert et al., 1981). Further studies by Bailey et al. (1980) suggested that supplemented phenylalanine improved the health status of birds fed diets containing ochratoxin A.

Lipids: Dietary high levels of oils (like olive, coconut, safflower) or animal fat, reduced mortality and in some instances improved body weight of chickens (Waldroup, 1997). The lipids extended their effects in part by interfering with absorption of the aflatoxins, i.e. the aflatoxin was not being absorbed from the high-fat diet (Smith et al., 1971).

Vitamins: Adequate vitamin supplementation is particularly important when feeds contain mycotoxins, because fungi can destroy vitamins in feeds. Interactions of aflatoxins with riboflavin, vitamin D, vitamin A and thiamin have been reported (Dickman et al., 1992). Recent work with fusarium toxicity (Waldroup, 1997) suggests that an antithiamin factor is a major toxic factor of F. proliferatum, and supplementation of diets with thiamin aided in ameliorating the toxicity of the mycotoxins in chick diets. The acute lethality of aflatoxin in rats was increased by a deficiency of vitamin A, while the carcinogenicity of aflatoxin was lessened by the deficiency (Rogers and Newberne, 1971). A diet deficient in vitamin D rendered chickens sensitive to growth inhibition by doses of aflatoxin too small to inhibit growth in a sufficient diet (Hamilton et al., 1974). Riboflavin and pantothenol had a sparing effect against aflatoxin when incorporated into the diets of ducklings (Lynd and Lynd, 1971). Dietary supplementation with biotin, which is involved in certain fatty liver syndromes, reduced in chickens the accumulation of lipid and retarded any change in the fatty acid profile of liver fat caused by aflatoxin (Bryden et al., 1979).

Antibiotics: Addition of 55 ppm of chlortetracycline to broiler diets helped to alleviate, but did not overcome the adverse effects of 10 ppm aflatoxin on body weight, feed conversion and mortality (Waldroup, 1997).

Antioxidants: Antioxidants aid in the overall detoxification process in the liver and in cells and thus may aid in alleviation of mycotoxicosis. Both vitamin E and selenium are involved in the formation of glutathione peroxidase, a compound vital in the cellular detoxification mechanism (Waldroup, 1997).

II- SECONDARY PREVENTION:

If the invasion of some fungi begins in commodities at early phase, several measures are suggested as follows:
Stop growth of infested fungi by re-drying the product (Suttajit, 2002).

Remove the contaminated seeds or grains. Removal of contaminated seeds, physical separation of infected grains is an efficient and feasible method of minimizing mycotoxins contamination. This is offered by either manual operation or with the help of an electronic sorter. Fungal infection of seeds or grains usually imparts characteristic colour or other physical properties (Goyal, 2002).

Protect stored products from any conditions which favour continuing fungal growth (Suttajit, 2002).

Decontamination or detoxification of mycotoxins contaminated products (Suttajit, 2002).

Inactivation of mycotoxins and decontamination or detoxification of commodities containing them can be classified as physical, chemical or microbiological:

I-Physical inactivation:

a-Thermal inactivation: Aflatoxins and most Fusarium mycotoxins are resistant to thermal inactivation for example, Fumonisine B₁ requires a high temperature (150- 200°C) to achieve 87-100% loss in corn (Chrevatidis et al., 2003b).

Boiling and autoclaving: Aflatoxins are heat-stable and are not totally destroyed by boiling, autoclaving and a variety of food and feed processing procedures (Christensen et al., 1977).

Heating and cooking under pressure can destroy nearly 70% of aflatoxin in rice compared to atmospheric pressure only 50% destroyed. Since aflatoxin resists to higher temperature up to 260°C, long-time cooking and overheating would destruct essential vitamins and amino acids in treated foods (Suttajit, 2002).

Roasting reduce the aflatoxin content of nuts, oil seed meals and corn (Marth and Doyle, 1979 and Conway et al., 1978) and its content in foods from 40-60% (FDA, 1979). Roasting temperature more than 250°C are necessary for effective aflatoxin degradation; increasing the moisture content of the substrate will enhance degradation (Weidenborner, 2001). Dry roasting of naturally contaminated peanuts produced a 40 to 50% loss of AFB1 and AFG1 and a 20 to 40% of AFB2 and AFG2, whereas microwave roasting of contaminated peanuts completely destroyed aflatoxins (Luter et al., 1982; Waltking, 1971). Roasting corn at 145 to 165°C reduced the aflatoxin B₁ by 4080% (Conway et al., 1978).

b-Storage of feed ingredients: Ochratoxin A can slowly breakdown merely during storage of grains and grain products as is documented by a decrease of more than 60% in naturally contaminated barley over a storage period of 2 years (Weidenborner, 2001).

c-Storage and physical treatments of milk may lead to some degradation of aflatoxin M₁, as follows:-

Storage: It is observed that AFM₁ in milk decreases with time. Approximately 40% of M₁ was lost after 4 days and about 80% after 6 days of storage at 0°C of AFM₁ contaminated milk (Applebaum et al., 1982). Storage at 5°C for 1-3 days caused the amount of M₁ to decrease by 11-25% (Kiermeier and Mashaley, 1977).

Freezing at -18°C for 30 and 120 days caused degradation of 14% and from 43 to 68% of M₁, respectively (Mckinney et al., 1973). Repeated freezing at -20°C and defreezing is also efficient in liquid medium to reduce the OA production (Deberghes et al., 1993).

Pasteurization reduces AFM₁ content of milk: Bulk pasteurization at 62°C for 30 min.
caused a reduction of 32% of M₁. High temperature short time processes of 72 and
80°C for 45 seconds, reduced the amount of M₁ content by 45 and 64%, respectively (Purchase
et al., 1972).

Sterilization of milk will cause a loss of some AFM₁. Processing of milk using steam pressure
of 1 kg/cm² for 20 min. with preheating and cooling for an additional 10 min. each, reduced
80% of M₁ (Purchase et al., 1972).

Concentration of milk to half its volume at
reduced pressure and 40°C lowered the content
of M₁ 64%. Drying with reduced pressure and
40°C resulted in 61% loss of the M₁
(Applebaum et al., 1982).

d-Sunlight: Sunlight degrades aflatoxin somewhat (FDA, 1979). Sunlight destroys 83% of the aflatoxin added to casein and 50% of that added to groundnut cake flour (Shantha and
Murthy, 1981). Exposure of aflatoxin contaminated groundnut oil to sun light has given very promising results as it destroyed about 99% of aflatoxins (Goyal, 2002).

e-Gamma irradiation: Exposure of milk to gamma irradiation (UV) for 60 minutes, resulted in 100% degradation of aflatoxin. Gamma irradiation is also efficient to prevent the production of OA or destroy it. Two to 3 kg and 4 to 5 kg are need for solid and liquid medium, respectively (Deberghes et al., 1993).

f-Wet and dry milling: Wet and dry milling processes, which are widely used for maize and
cereal grains, have been shown to result in reduced mycotoxin levels (zearalenone, fumonisins, aflatoxins, trichotheecenes and ochratoxin A) in several fractions such as milling solubles, gluten, fiber, starch and germ (Lopez-Garcia and Park, 1998). Wet milling of maize, produces starch free, or almost free of zearalenone, fumonisins and aflatoxins, but T-2
toxin is increased in maize germ (Riley and
Norred, 1999). During dry milling of maize, high levels of zearalenone were found in the maize germ, degermer fines, bran meal, hull and high fat fractions and low levels (10-22%) occurred in the prime products (grits, low-fat meal and flour). Dry milling caused an accumulation of fumonisins in the bran, germ and fines fractions that are widely used in the production of animal feeds. Increasing refinement of maize meal caused lowered fumonisin levels by as much 95% in the fine maize meal compared to maize screenings. During experimental wet-milling of maize the maize bits (starch, fiber and gluten) contained most of the OA (51%) of all the maize farctions (Weidenborner, 2001).

h-Adsorbents: Addition in the diet of nutritionally inert adsorbents such as hydrated sodium calcium aluminosilicate, activated carbon, bentonite, clays and special polymers reduces the absorption of mycotoxins from the gastrointestinal tract thus avoiding or/ reducing the toxic effects for livestock and their carry over of mycotoxins into animal products (Chrevatidis et al., 2003d).

Hydrated sodium calcium aluminosilicates (HSCAS) are a phyllosilicate clays which are very effective with regard to preventing aflatoxicosis in a great variety of animals including chickens, turkey poults, goats, cows, pigs and lambs (Ramos et al., 1996). HSCAS has been shown to adsorb AFB₁ with high affinity and high capacity in the aqueous solutions including milk, in contaminated oils, diminish
the effects of aflatoxins in young animals and decrease the level of AFM1 in milk from lactating dairy cattle and goats (Lopez-Garcia et al., 1999). The efficacy of HSCAS was quite limited against zearalenone and OA and totally ineffective for trichothecenes such as T-2 toxin, diacetoxyscripenol and deoxynivalenol (Chrevatidis et al., 2003d).

Activated carbon (AC) has also been studied for its ability to bind aflatoxins in vivo and in vitro (Lopez-Garcia et al., 1999). AC reduces dietary conversion of AB1 to AM1 in cows (Marquez et al., 1993). AC is also quite effective in absorbing OA from aqueous solutions but has no beneficial effect when tested in vivo (Rotter et al., 1989).

Sodium bentonite has been used as a binding and lubricating agent in the production of pelletted feeds. Dietary addition of bentonite or zeolite have shown to alter the effects of T-2 toxin and zearalenone (ZEA) in rats (Carson, 1982 and Smith, 1980).

Cholestyramine (CH), a resin used for pharmaceutical purposes in decreasing total and LDL cholesterol, adsorbed almost 100% of ZEA from gastric and intestinal fluids.

In particular 1 gm of CH was able to adsorb up 2mg of ZEA (Ramos et al., 1996). In studies on rats CH was tested as a protective agent against OA and was found to be able to decrease the concentration of OA in plasma, the excretion of OA and its metabolites in urine and bile and to increase OA excretion in feces (Kerkadi et al., 1998).

II-Biological detoxification:

Microbial strains including yeast, molds and bacteria, have been seened for their ability to modify and/or inactivate aflatoxins, deoxynivalenol, zearalenone, T-2 toxin and other trichothecenes, but their practical use has not been shown (Chrevatidis, 2003b; Ciegler et al., 1966). Flavobacterium aurantiacum was reported to significantly remove aflatoxin from a liquid medium without producing toxic by-products (Ciegler et al., 1966). It removes AFB1 form milk, maize, peanuts and soybeans, while AFG1 and AFM1 are also metabolized (Weidenborner, 2001). Black yeast fungus and a bacterium strain have been found to hydrolyze fumonisin B1 to aminopentol and tricarboxylic acid. The same microorganisms seem to be able to further detoxify aminopentol with release of CO2 (Chrevatidis, 2003 b).

Lactic acid bacteria and yeasts expressing mycotoxin degrading enzymes may offer a natural way of providing these activities in fermentation process (Chrevatidis, 2003b). Fermentation with yeasts has also effective in destroying patulin and rubratoxin B (Lopez-Garcia and Park. 1998). Probiotic mixtures of Lactobacillus and Propionibacterium may reduce bioavailability of dietary AF (Ahokas et al., 1998). Fermentation of contaminated grains, resulted in degradation of aflotoxins (Dam and Satterlee, 1977).

Some mycotoxins are detoxified during ensiling and other fermentation processes (aflatoxins, mycophenolic acid and patulin) while others are able to degrade or transform their own products under suitable conditions (Karlovsky, 1999). Ensiling contaminated high-moisture corn did not adequately degrade aflatoxins. It was postulated that insufficient acid was produced by this procedure to catalyze the transformation of aflatoxin B1 to aflatoxin B2, (Lindenfelser and Ciegler, 1970).

III-Chemical detoxification:

Aflatoxins are crystalline substances, freely soluble in polar solvents such as chloroform, methanol and dimethyl sulfoxide and dissolve in
water to the extent of 10-20 mg/L. Crystalline aflatoxins are extremely stable in the absence of light and practically UV radiation, even at temperatures in excess of 100°C. A solutions prepared in chloroform, or benzene is stable for years if kept cold and in the dark. The lactone ring makes them susceptible to alkaline hydrolysis, and processes involving ammonia or hypochlorite have been investigated as means for their removal from food commodities, although questions concerning the toxicity of the breakdown products have restricted the use of this means of eradicating AFs from food and animal feeds. If alkaline treatment is mild, acidification will reverse the reaction to reform the original aflatoxin. In acid, AFB₁ and G₁ are converted to AFB₂α and G₂α by acid catalytic addition of water across the double bond of the furan ring. Oxidizing reagents react with and the molecules lose their fluorescence properties (Chrevatidis et al., 2003a).

Many chemicals have been tested for their ability to degrade or inactivate aflatoxins and some mycotoxins including acids, bases, aldehydes, bisulfite, oxidizing agents and various gases.

A-Bases: Many bases are tested for decontamination of mycotoxins such as ammonia, calcium or sodium hydroxide, calcium hydroxide plus monomethylamine and sodium bicarbonate.

Ammoniation: Either anhydrous ammonia, NH₃ (gas) or ammonium hydroxide, NH₄OH (aq:ammonia=liquid), can be used at levels of 0.5 to 1.5 percent of the dry weight of feed like corn. The lower rate (0.5 percent) would be appreciated when the corn is 16 percent moisture or more, the temperature is 80 degrees F or more and the aflatoxin levels are less than approximately 200 ppb. The 1.5 percent rate should be used for high levels of aflatoxin and/or when the temperature and moisture conditions are less lowered (PER, 2003; USDA, 1998). The method consisted of injection or spraying ammonia onto contaminated feed, storing in air-tight containers e.g. plastic bags for about 3 weeks. The feed is heated after storing in oven at 50°C for 24 hours to eliminate the residual ammonia and finally aerated to remove some of the ammonia odor and makes the feed more acceptable for animals (Marquez et al., 1993).

Ammoniation yielded from 79-90 percent reduction in aflatoxin B₁ in contaminated feed. Ammonia reacts with the aflatoxin molecule by breaking an oxygen bond making the resulting products far less toxic (PER, 2003). Ammonia causes lactone ring opening of AFB₁, ultimate splitting off of the cyclopentone part. Several breakdown products of AFB₁ have been identified e.g. AFD and the 206 molecular weight compound. Both substances showed a 450-fold decrease in mutagenicity compared to AFB₁ (Weidenborner, 2001). Ammoniation, a procedure used for decontamination of aflatoxins, yielded a 79% reduction in fumonisin B₁ levels in naturally contaminated corn. Ammoniation of ochratoxin A-containing grain is effective in eliminating its toxicity (Marquardt and Frohlich, 1992).

Since ammonia vapor is only 60 percent as heavy as air, it should be applied under the corn. Corn with moisture content below approximately 15 percent, will need rewetting so that the ammonia will be absorbed. Up to 3 percent moisture can be added to the surface of the corn by spraying with water. Let the corn absorb the water for 6-8 hours before treating with ammonia. The warmer the corn, the faster the ammonia reaction. Corn with a moisture content of at least 15 percent and at a temperature of 90 degrees F can be treated in approximately 7 days, 75 degrees F corn
requires 14 days. Corn with a lower moisture content and/or temperature will require a treatment time of 21 days. When ammonia vapor is absorbed by the corn the temperature will increase 10 to 20 degrees (USDA, 1998).

Ammonium hydroxide (NH$_4$OH) is usually about 29 percent ammonia (by weight) and can be sprayed directly on corn. The application of ammonium hydroxide will also add about 2 percent moisture to the corn for every percent of actual ammonia used. This may eliminate the need for rewetting dry corn (PER, 2003).

Precautions for handling and application of ammonia:

Procedures involving anhydrous ammonia are less costly, but may be hazardous because of toxic fumes and the danger of explosions (Duncan and Hagler, 1986).

Ammonia in concentration greater than 1 percent is corrosive to metal surfaces. Ammonia also discolors and darkens treated corn kernels (as the sugars are carmelized) and increases its temperature about 10-20 degrees at the time of treatment. Applicators should use the following precautions when using ammonia (USDA, 1998; PER, 2003):

- When handling corn with high levels of aflatoxin, always wear a dust mask. The contamination dust is hazardous.
- Applicators should wear protective goggles, neoprene gloves and long sleeves (ammonia is extremely caustic and cause skin burns and respiratory and eye injuries).
- A large supply of water should be close in hand for flushing of eyes or skin if an accident occurs.
- Workers should not enter bins in which it is being applied to avoid breathing ammonia. Prolonged inhalation can cause suffocation and death.

- Do not allow any spark of flame in areas where ammonia is being used.
- Detoxified corn must be thoroughly aerated in order to prevent feed refusal or reduced feed intake due to residual ammonia (Duncan and Hagler, 1986).

Calcium hydroxide [Ca(OH)$_2$]: Detoxification of AFB$_1$ with calcium hydroxide [Ca(OH)$_2$] or calcium hydroxide and monomethylamine (CH$_3$-NH$_2$), simultaneously resulted in 94-100% destruction of AFB$_2$. The mechanism of destruction is the opening of the lactone ring and decarboxylation. The detoxification process consisted of adding Ca (OH)$_2$ to the dry diet at a concentration of 2% (W/W), mixing for 5 minutes and adding monomethylamine at a concentration of 0.5% and water to adjust moisture content to 24%. Mixing in a closed environment (in a mixer equipped with a steam jacket) for 1 hour at 100°C and finally dry in a convection oven at 40°C to remove volatile compounds (Park et al., 1983).

Treatment of FB1-contaminated corn (100 mg/kg) simulating modified nixtamalization (heat treatment with NaHCO$_3$ + H$_2$O$_2$ alone or with Ca(OH)$_2$ gave 100% reduction of FB1, whereas the traditional nixatamalization (treatment with Ca(OH)$_2$ only) was not effective because it produces hydrolyzed FB1 which is still toxic (Park et al., 1996).

Calcium hydroxide monomethylamine: Calcium hydroxide monomethylamine has been used to decontaminate feeds containing T-2 toxin and diacetoxyscirpenol at 10 to 20 mg/kg, the success of the producer is dependent on the moisture content of the feed and the processing temperature. In particular about 50% of mycotoxin reduction was observed when the treatment was performed at about 25°C and 10% moisture in 4 hours; when the moisture content was increased to 25%, T-2 toxin level
was reduced by 95 to 99% (Chrevatidis et al., 2003 c).

Sodium hydroxide (NaOH) and sodium bicarbonate (NaHCO₃): The mixing of diet with NaOH or NaHCO₃ at a role of 100 ml of 5N base per Kg diet and heating the mixture in an oven for 1 hour at 100°C, then storing at room temperature for 7 days, causes 67 and 71% destruction of AFB₁ (Mashaly et al., 1983).

Sodium bisulfite: Sodium bisulfite treatment of grain results in the greatest reduction in deoxynivalenol (Young et al., 1986). Sodium bisulfite (a common food additive) has been shown to react with aflatoxin (B₁, G₁, M₁ and aflatoxicol) to form sulfonate derivatives (Chervatidis et al., 2003C). Sodium bisulfite solutions are capable to reduce DON level (85%) in contaminated corn (4.4 mg/kg DON) and form a DON-sulfonate conjugate when the treatment was performed at 80°C for 18 hours (Young et al., 1987).

B-Acids: Acids effectively convert AFB₁ and AFG₁ to their corresponding hemiacetal forms AFB₂ and AFG₂ (Weidenborner, 2001). Acetic (C₂H₅OH), citric, lactic and phosphoric acid (H₃PO₄) are recorded as a mycotoxin detoxificating agents.

Acids are used at a concentration of 100ml of 5N acid per Kg diet. The detoxification process consisted of adding acid to the diet, mixing and heating in an oven for 1 hour at 100°C. After storing at room temperature for 1 week the process causes reduction in aflatoxin B₁ in diet 88, 57, 80 and 68% for acetic, citric, lactic and phosphoric acid, respectively (Mashaly et al., 1983). Formic, propionic and scorbic acids are found to degrade OA at concentrations from 0.25% to 1% after exposure of 3-4 hours (Chrevatidis et al., 2003 c).

c-Oxidizing agents: Oxidizing agents such as sodium hypochlorite (NaOCl), hydrogen peroxide (H₂O₂) and (HCHO) are tested for detoxification of mycotoxins. Treatment of diet with NaOCl, H₂O₂ or HCHO at level of 100ml (0.02%) of antioxidant per Kg diet and storing at room temperature for 7 days produced 82, 78 and 62% destruction of AFB₁, respectively (Mashaly et al., 1983).

Incubation of contaminated feed with 0.05% H₂O₂ for 30 minutes at room temperature causes 100% destruction of citrinin (Fouler et al., 1994). The toxicity of ochratoxin A in contaminated diet can be reduced by heating of diet with H₂O₂ at 100°C in an alkaline condition.

D-Gasses: It is recorded that the treatment of contaminated corn with ozone gas provided almost complete protection against the toxicity of aflatoxins in young turkey poults. Ozonation degrades and detoxifies aflatoxins in naturally contaminated maize promising (Riley and Norred, 1999). Dwarakanath et al. (1968) reported reduction of aflatoxin levels in cotton seed and peanut meal by ozone treatment under various conditions of moisture, temperature and time.

III-TERTIARY PREVENTION:

Once the products are heavily infested by toxic fungi, the primary and secondary prevention would not be then feasible. Any action would not be effective as the practices mentioned above, and reduce toxin formation (Suttajit, 2002). Only a few practices are recommended:-

-Complete destruction of the contaminated products.
-Detoxification or destruction of mycotoxins to the minimal level.
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الوقاية والتخلص من السموم الفطرية

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السموم الفطرية هي مركبات كيميائية سامة تفرزها أنواع من الفطريات التي تنمو على المنتجات الغذائية.

وتختلف السموم الفطرية باختلاف تركيبها الكيميائي وتأثيرها السمى ودرجة ضرورتها.

ويؤدي تغذية الحيوانات والطيور على علانات تحتوى على مستويات مرتفعة من هذه المركبات إلى أعراض مرعبة مثل ضعف النمو، انخفاض معدل إنتاج اللحم واللبن والبيض، ضعف الجهاز المناعي وارتفاع الإصابة بالأمراض وارتفاع نسبة النفوق، كما تؤدي متبقيات هذه المركبات إلى المنتجات الحيوانية إلى أضرار بالغة مثل السرطانات في الإنسان.

لذا يجب مراقبة المواد الغذائية لأي نموات فطرية واختبارها دورياً لمعرفة مدى تواجد أي من السموم الفطرية.

وتركزها بالإضافة إلى المعدلات المسموح بها لكل نوع من السموم وكل حيوان.

وتتطلب الإجراءات الوقائية للسموم الفطرية في تغذية الحبوب جيداً بعد حصادها، وخفض نسبة الرطوبة في المنتجات الغذائية وتخزينها في ظروف ملائمة وضمان مضايقات الفطريات لها، والتخلص من السموم الفطرية في حالة تواجدها بنسب مرتفعة في المنتجات الغذائية يمكن استخدام أي من الطرق الآتية مثل فصل الحبوب الموجودة عليها نموات فطرية واضحة، تحميص الحبوب المصابة، التخلص من السموم الفطرية بيولوجياً باستخدام بعض أنواع النيكروبات، أو استخدام العلاجات الكيميائية مثل الفلوريد والأحماض. وقد استهدفت هذه الورقة إقامة الضوء على العديد من الطرق المستخدمة في الوقاية والتخلص من السموم الفطرية في المنتجات الغذائية بالأهداف.