ADVERSE BIOLOGICAL EFFECTS OF AFLATOXIN TOXICITY

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Introduction

Fungi have benefited man over the years but on the other hand they produce highly poisonous secondary metabolites called 'mycotoxins' that cause illness or death when ingested by human beings or animals. Aflatoxins are mycotoxins which have toxic and carcinogenic effects in different species of livestock including poultry. They are produced primarily by toxigenic strains of Aspergillus flavus and Aspergillus parasiticus which are distributed in soil and air particularly under tropical conditions. Four major aflatoxins are aflatoxin B_1 (AFB_1), Aflatoxin B_2 (AFB_2), Aflatoxin G_1 (AFG_1) and Aflatoxin G_2 (AFG_2). There are fourteen other aflatoxins known, most of which are metabolites formed endogenously in animals which consume one or more of the major aflatixins. Metabolites of toxicological significance include AFB_1, AFB_1 2, 3 oxide, AFM_1, aflatoxicol and AFB_2.

Aflatoxins and their metabolites affect primarily liver causing various dysfunctions which involve interference with various biological processes like DNA replication, RNA synthesis, protein, lipid and carbohydrate metabolism, hence, causing loss of health and productivity in man and animals. So, to eliminate or minimize the ill-effects of aflatoxins, it is necessary to regulate or check the aflatoxin levels in foods and feeds. Care should be taken to minimize contamination and check the growth of toxigenic fungi at various stages of plant growth, processing and storage of products. Different methods (physical, chemical and biological) to detoxify aflatoxins, economic implications of aflatoxin contamination in feeds and foods have also been discussed.

Occurrence

Aflatoxin producing strains of Aspergillus are distributed worldwide in soil and air. Aspergillus species affect three major feedstuffs: corn, cottonseed and peanuts during growth, harvest, processing, transportation and storage when the moisture (>14%) and temperature (at least 25°C) conditions are favourable. Aflatoxin profile also depends on the substrate (feed or seed) and the type of mold involved.

When these requirements are met, mold infestation followed by aflatoxin formation in target feedstuffs is likely to occur. These feedstuffs represent a major source of nutrients fed to all classes of livestock and poultry. Aflatoxin producing fungi are more commonly found in tropical countries like India where both temperature and humidity are favourable for their growth.

Adverse biological effects due to aflatoxin toxicity

Aflatoxins are the subject of extensive study

Table 1: Effect of Aflatoxins on Performance of Different Species

Species	Observed Effects
Cattle	Reduced growth rate in calves and fattening cattle, unthriftiness, drop in milk yield, contamination milk scours, haemorrhage.
Pigs	Reduced growth rate in piglets and finishing pigs, jaundice, haemorrhage, increased susceptibility to infectious diseases
Poultry	Reduced growth rate in broilers, depressed egg production, increased susceptibility to disease and haemorrhage.
Sheep	More resistant than other livestock to aflatoxin production

because of their acute and chronic toxicity in most of the animal species. The effects of aflatoxins on animal health vary from species to species (Table 1). Calves, chicks, ducklings, and pigs are susceptible to aflatoxin B_1 while goats, rats and sheep are comparatively resistant (Patterson and Allcroft, 1970). Among the poultry species, ducklings are more susceptible followed by turkey, poults, phesants, chicks, mature chicken and quails in that order. The LD_{50} values have been used to determine the susceptibility to acute aflatoxicosis (Table 2). LD_{50} values depend on several factors like age, sex, strain, condition of the animal, rate of administration, composition of diet and time lapse before measurement of LD_{50}. The most potent carcinogen is AFB_1 followed by AFM_1, AFG_1, and AFG_2 in the order of decreasing potency. Various physiological and environmental factors affect the susceptibility to aflatoxin induced carcinogenicity through alterations in metabolism. Aflatoxins have mutagenic activity too.

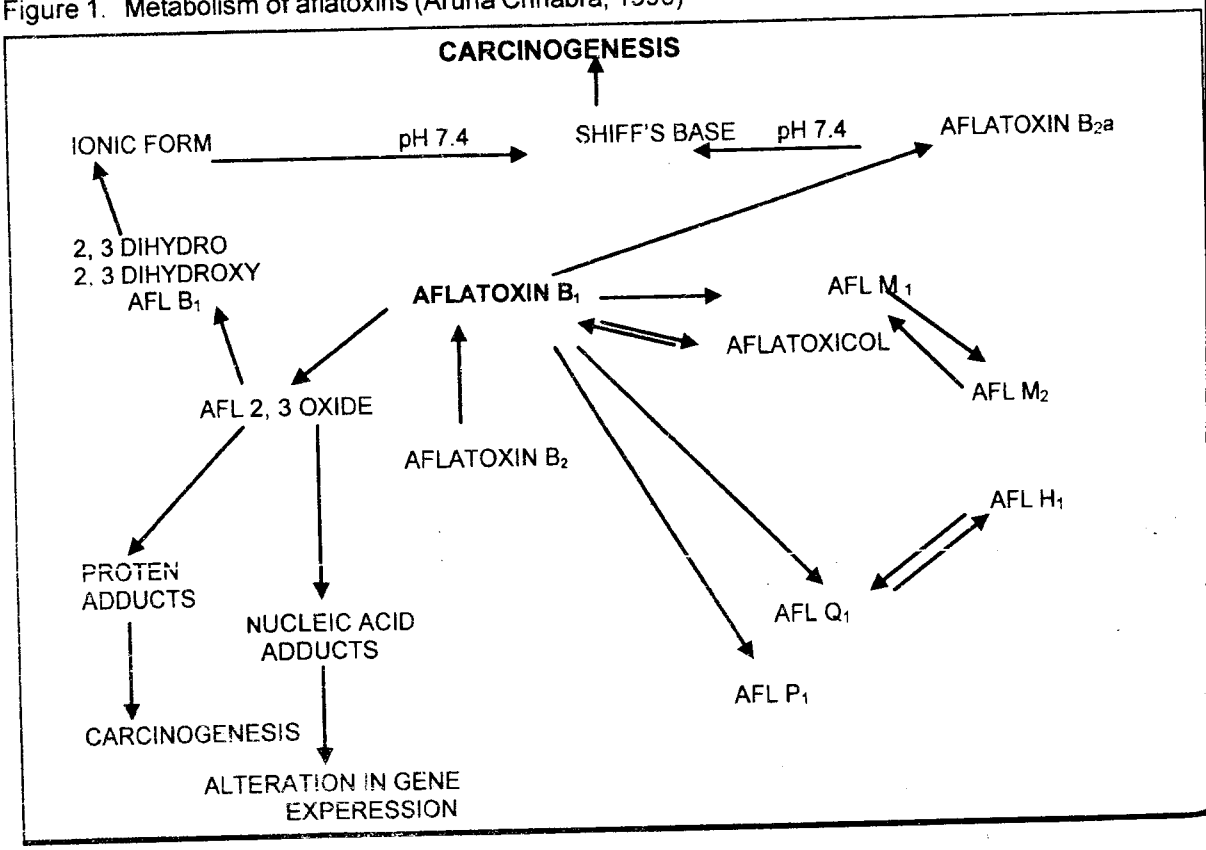
AFB_1 and aflatoxicol are the most potent mutagens. Chromosomal aberrations indicate the mutagenicity of AFB_1. Through it is inconclusive whether aflatoxins are teratogenic, birth defects like fetal resorption have been observed in hamsters.

Liver is the primary target organ in all the species because of its direct involvement in the metabolism (Fig.1). Numerous liver functions are affected and cumulative effects can be fatal to animals. As the functions of liver are affected, other metabolic disturbances such as derangement of blood clotting mechanisms, jaundice and reduction

Table 2: LD_{50} (mg/kg Body weight) of AFB_1 for Various Animal Species (Patterson and Allcroft, 1970)

Species	LD_{50} value
Ducklings	0.3 - 0.5
Rabbit	0.3 - 0.5
Rainbow trout	0.5
Pig	0.62
Dog	1.0
Chicken	6.16
Mouse	9.0

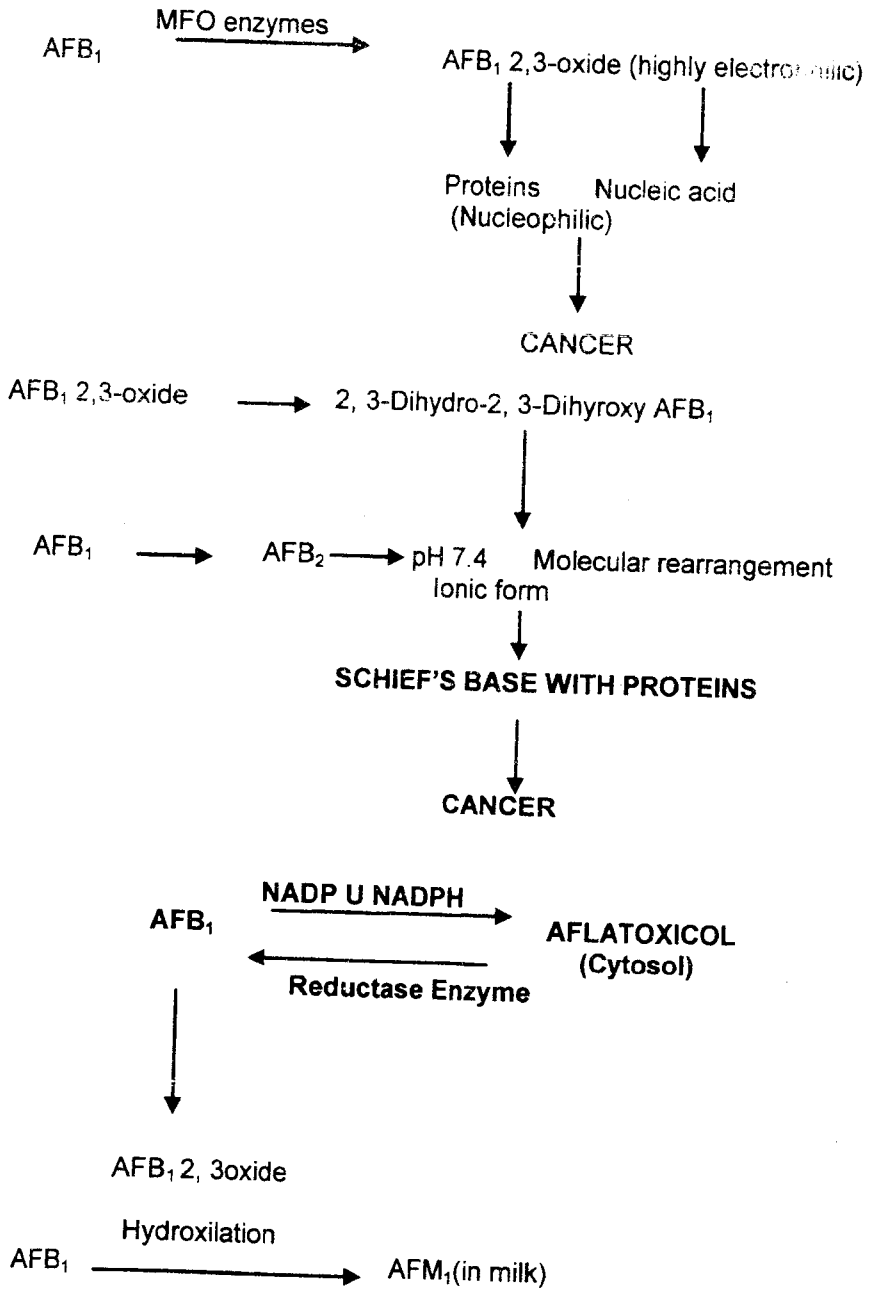
Figure 1. Metabolism of aflatoxins (Aruna Chhabra, 1995)



of essential serum proteins which are synthesized in the liver occur. In addition to liver damage, higher doses may cause necrosis of renal tubular epitheliurn in rats, dogs, guinea pigs and monkeys (Newberne and Rogers, 1981).

Aflatoxin induced tumors of organs other than liver have also been reported (Wogan, 1973). Carbohydrate, lipid and protein metabolism are disrupted when aflatoxins interact with key enzymes. They cause reduction in blood glucose and glycogen content of brain and liver (Raj et al, 1970). Accumulation of lipids occurs in the liver (fatty liver) and inhibition of fatty acids and cholesterol biosynthesis occur when aflatoxin interferes with lipid

Figure 2. Metabolic fate of afb_1 and transfer to products



metabolism. Aflatoxins inhibit protein synthesis. DNA and RNA synthesis in liver is also adversely affected due to high affinity of aflatoxin for them (Raj et al, 1970; Gelboin et al, 1966 and Wragg et al, 1967). Aflatoxin B_1 tends to reduce vitamin A concentration in liver. They exert influence on resistance to infection and development of immunity (Pier, 1973) and result in decreased growth rate, lowered productivity (milk and eggs) and immuno-suppression which impair native resistance to infection thus affecting the animal's ability to defend against invading pathogens. Chronic aflatoxicosis interferes with the development of acquired immunity, the serious consequence of which will reduce effectiveness of vaccination normally carried out in pigs and poultry.

Metabolic fate and transfer to products

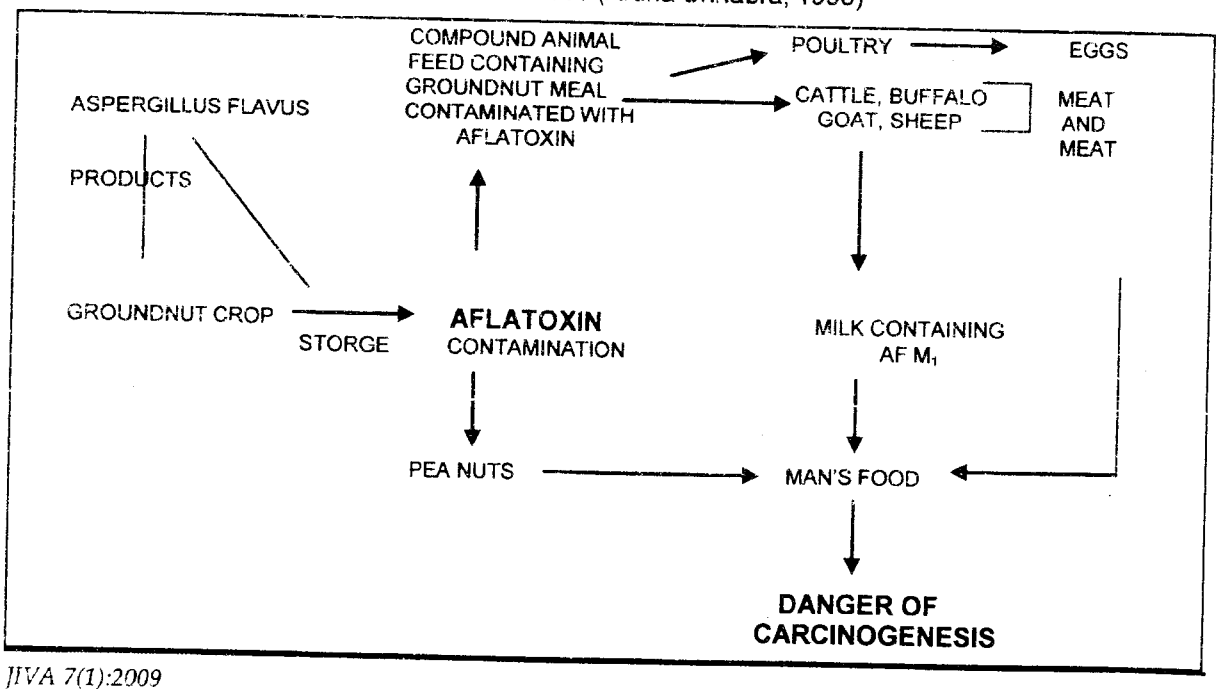
There are four metabolic reactions (Fig.2) of toxicologic significance in animals (Cheeke and Shull, 1985). The metabolic fate of aflatoxins in livestock and poultry is highly pertinent for two reasons: (i) Metabolism (activation) accounts for much of the extreme toxicity and carcinogenicity of aflatoxins, (ii) Distribution of aflatoxin or their metabolites to various body locations of food animals can impart hazardous residues to products (milk and eggs) used as human food (Fig. 3).

The principal route of aflatoxin excretion is the faeces (>= 75%). The remainder (<= 20%) is excreted in the urine. Only a small portion of AFB_1 administered orally is found unaltered in excretions (faeces and urine) or secretions (milk and eggs) indicating extensive metabolism.

Dairy cows consuming rations contaminated with aflatoxin excrete the aflatoxin metabolite (AFM_1) in milk in concentration, which is related to feed aflatoxin. AFM_1 holds significance because of its inherent carcinogenicity. It is considered as an important food chain contaminant and a major food safety concern. In general, the ratio of level of AFB_1 in feed to that milk is 300:1 (Rodricks and Stoloff, 1977). However, the conversion varies with feed concentration and individual cow. AFM_1 excreted in milk is later carried to milk products, i.e. butter, dried milk, cheese, infant formula etc. (Nikov et al., 1991).

The carcinogenic nature of aflatoxins and their involvement in animal and human diseases makes it necessary to stipulate maximum levels of aflatoxins that can be permitted in food and feeds. What ever the limits may be, it is important that the method by which the level of aflatoxin is estimated is accurate, keeping in view its effect on the health of human beings and livestock or to make the product acceptable in the international market.

Figure 3. Mode of entry of aflatoxin in human food (Aruna Chhabra, 1995)



Regulation of aflatoxins

Aflatoxins may enter the food supply by direct contamination resulting from mold growth on the feed/ food or by indirect contact through use of contaminated ingredients in processed feed/food. Indirect exposure also results from consumption of animal products such as milk, eggs, meat, fish which have aflatoxins residues. Several countries have developed legislation to regulate and control aflatoxins. The actual tolerance levels of aflatoxins are.

- Aflatoxin B_1 in foodstuffs – 5pp.
- Sum of aflatoxinsB_1, B_2, G_1, G_2 in foodstuffs is- 10 and 20 ppb.
- Aflatoxin M_1 in milk – 0.05ppb.
- Aflatoxins in feedstuffs for dairy cattle- 10ppb.

Tolerance levels of aflatoxin established by Food and Drug Administration, USA (VanEgmond, 1989) are:

- Aflatoxin in foods – 20 ppb (expected to be lowered to 15ppb)
- Tolerance level of aflatoxin in milk and milk products - 0.5 ppb (also tolerance level).
- Tolerance level for cottonseed meal, corn and mixed feed for beef cattle –300 ppb.
- Tolerance level for feds used for breeding cattle, breeding swine and mature poultry-100ppb.

The differences between the limits of different countries sometimes are substantial which makes international harmonization of aflatoxin regulation highly desirable.

Control and detoxification

Aflatoxins are natural contaminants of food and feed and in many instances are unavoidable. The growth of this toxigenic mold can be controlled in the food products by taking adequate precautions during its processing and in feed during harvesting and storage. The best approach to eliminate aflatoxins from foods is to prevent mold growth at all levels of production, harvesting, transportation and storage of food/feeds. This involves prevention of insect damage and mechanical damage to agricultural commodities as well as moisture control at levels which do not permit mold growth and storing at temperatures and under conditions which minimize mold development. Control also involves quality control procedures to detect and remove contaminated products from commercial channels before they reach the consumer. Total control of aflatoxin contamination is almost impossible.

A food /feedstuff containing a toxic substance might be rendered biologically harmless by completely removing the toxin, converting the toxin to a non-toxic derivative and degrading the toxin to simpler, inactive products which can be achieved by the following three detoxification methods.

Physical Methods

- Hand picking or Electronic sorting
- Density separation for floatation
- Adsorption-cum-filtration technique
- Heat treatments
- Exposure to UV light (irradiation)

Chemical Methods

- Extraction with organic solvents like chloroform, benzene, methanol.
- Use of adsorbants like carbon, bentonite, clays and aluminosilicates.
- Use of strong acids like hydrochloric acid.
- Use of strong bases like ammonia, sodium hydroxide.
- Use of oxidizing agents like hydrogen peroxide, ozone.
- Use of bisulphite like sodium bisulphite
- Use of chlorinating agents like sodium hypochloride, chlorine dioxide.
- Use of propionic acid.

No single treatment is completely successful in degrading/removing toxins and simultaneously retaining nutritional and functional quality of the treated commodity. Ammoniation appears to be the most promising treatment for inactivation of aflatoxin in feedstuffs although it would be of limited value for the removal of aflatoxins from feeds. Bisulphites and

adsorbants are the best treatments for eliminaton of aflatoxin from foods. Propionic acid and sodium bisulphite pretreatment control aflatoxin in feeds (groundnut cake) by preventing fungal growth (Ghosh et al, 1996).

Biological Methods

♦ Use bacteria like Flavobacterium aurantiacum to degrade aflatoxin or Lactobacillus delbrukii to transfer it into less toxic products like aflatoxicol and aflatoxinB2a.

♦ Use of fungi like Aspergillus niger, Trichoderma viridae, atoxigenic strains of A. flavus and A. parasites

♦ Use of onion oil at different concentrations (10, 200 and 500ppm) to reduce fungal growth and aflatoxin production by A.flavus and A.prasiticus (Zohri et al, 1995).

The above-mentioned atoxigenic strains of A. flavus and A. parasiticus are useful in biological control of aflatoxin contamination of cottonseed and maize. They reduce both pre-harvest and post-harvest contamination (Brown et al, 1991). Microbiological aflatoxin detoxification methods that transform aflatoxin B_1 to less toxic derivatives as given below though promising need further investigation.

Conclusion

Aflatoxins have an adverse impact on national economy. They invade food crops and the most affected are corn, peanuts, sorghum, cottonseed, wheat and other grain crops. Fruits, vegetables and animal products also get contaminated by it resulting in unbearable economic losses to farmers and exporters as the food remains unfit for consumption. Additional costs are spent to control it. There is an increase in import of food and feeds which ultimately results in an imbalanced national economy. Since loss of animal and human life due to consumption of contaminated feed and food is the ultimate cause of concern, proper care to reduce or to eliminate aflatoxins at every possible step must be taken.

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