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AN OVERVIEW OF AFLATOXICOSIS OF POULTRY: ITS CHARACTERISTICS, PREVENTION AND REDUCTION

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ABSTRACT

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Aflatoxicosis represents one of the serious diseases of poultry, livestock and other animals. The cause of this disease in poultry and other food-producing animals has been attributed to the ingestion of various feeds contaminated with A. flavus. This toxigenic fungus is known to produce a group of extremely toxic metabolites, of which aflatoxin B_1 (AFB_1) is most potent. Avian species especially chicks, goslings, ducklings and turkey poults are most susceptible to AFB_1 toxicity. The toxic effects of AFB_1 are mainly localized in liver as manifested by hepatic necrosis, bile duct proliferation, icterus and hemorrhage. Chronic toxicity in those birds is characterized by loss of weight, decline in feed efficiency, drop in egg production and increased susceptibility to infections. The incidence of hepatocellular tumors, particularly in ducklings, is considered to be one of the serious consequences of aflatoxicosis. Even though prevention and avoidance are the best way to control aflatoxicosis, natural contamination of crops with A. flavus is sometimes unavoidable. Such aflatoxin-contaminated feeds can be decontaminated using various methods which mainly focus on physical removal or chemical inactivation of the toxins in the feeds. Moreover, dietary additives such as activated charcoal, phenobarbital, cysteine, glutathione, betacarotene, fisetin and selenium have also been reported to be effective in the reduction of aflatoxicosis in poultry.

INTRODUCTION

The aflatoxins are a group of extremely toxic metabolites produced by the common molds Aspergillus flavus and parasiticus. These fungi are ubiquitous and as a result likelihood of their contaminating foodstuffs and animal feeds is quite high. The occurrence of aflatoxins in agricultural commodities depends on such factors as region, season and the conditions under which a particular crop is

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grown, harvested or stored. Crops grown under warm and moist weather in tropical or subtropical countries are especially more prone to aflatoxin-contamination than those in temperate zones. Groundnuts and groundnut meal are by far the two agricultural commodities that seem to have the highest risk of aflatoxin contamination (Wyllie and Morehouse, 1977; Patterson, 1983). Corn, cottonseed, Brazil nuts, copra, various tree nuts, and pistachio nuts are the other commodities quite susceptible to the invasion of aflatoxin-producing fungi. Although these commodities are important as substrates, the moisture content of the substrate and temperature are the main factors regulating the fungal growth and toxin formation. A moisture content of 18% for starchy cereal grains and 9-10% for oil-rich nuts and seeds has been established for maximum production of the toxin (WHO, 1979). On the other hand, the minimum, optimum and maximum temperatures for aflatoxin production have been reported to be 12°, 27°, and 40-42°C, respectively (Christensen and Nelson, 1976).

Frequent contamination of corn and other commodities with high levels of aflatoxins has been a serious problem all over the world resulting in significant economic losses to farmers and a health hazard to farm animals and humans as well. Farm animals are susceptible to aflatoxin poisoning to a varying degree. For most species, oral LD50 values of aflatoxin B_1 vary from 0.03 to 18 mg/kg body weight (Table 1). Among the food-producing animals, chick embryo, goslings, ducklings and turkey poults have been reported to be most susceptible as opposed to female rats being most resistant (Newberne, 1974; WHO, 1979; Cavalheiro, 1981; Malkinson et al., 1982). Furthermore, within poultry, certain breeds appear to be more sensitive than others. Exposure of chickens to as low as 0.2-1 ppm of aflatoxins in diet has been shown to result in poor growth rates, reduced feed efficiency, marked drop in egg production, liver damage, bile duct proliferation and most importantly decreased resistance to common infectious diseases including coccidiosis (Smith and Hamilton, 1970; Newberne, 1973; Pier and Heddleston, 1970). This has led to a public concern expressed not only about the effects that aflatoxin-contaminated feeds may have on the growth and health of poultry, but also about the possible transmission of toxic residues into meat and eggs resulting in a potential hazard to human health. The purpose of this paper is to discuss important characteristics of acute and chronic toxicity of aflatoxins, parti-

TABLE 1

Comparative LD50 or lethal values for aflatoxin B_1

Species	Oral LD50/Lethal dose[a] (mg/kg)
Chick embryo	0.025
Duckling	0.3
Turkey poult	0.5
Chicken, New Hampshire	2.0
Chicken, Rhode Island	6.3
Sheep	5.0
Rat (male)	7.2
Rat (female)	17.9
Rabbit	0.3
Cat	0.6
Pig	0.6
Guineapig	1.4
Hamster	10.2
Mouse	9.0
Baboon	2.0

[a]Ref: Edds, 1973; WHO, 1979.

cularly aflatoxin B_1, in poultry and to review available literature concerning ways to prevent and reduce the aflatoxicosis eventually leading to significant economic gains and improvement in the wholesomeness of animal and human food.

TYPES OF AFLATOXINS

Aflatoxins are polycyclic, unsaturated compounds consisting of a coumarin nucleus flanked by a highly reactive bifuran system on one side and either a pentanone (characteristic of B series) or a six-membered lactone (characteristic of G series) on the other (Figure 1). Although 17 aflatoxins have been isolated (WHO, 1979), only 4 of them are well known and studied extensively from toxicological point of view. Being intensely fluorescent in ultraviolet light the four are designated by letters B_1, B_2, G_1 and G_2 representing their blue and green fluorescence in UV light. Two other familiar aflatoxins, M_1 and M_2, are in fact metabolites of B_1 and B_2 and labelled so because of their presence in milk of animals previously exposed to B_1 and B_2. Of all the above-named aflatoxins, aflatoxin B_1 (AFB_1) is the most acutely toxic to various species, some being highly susceptible while others relatively resistant (Table 1).

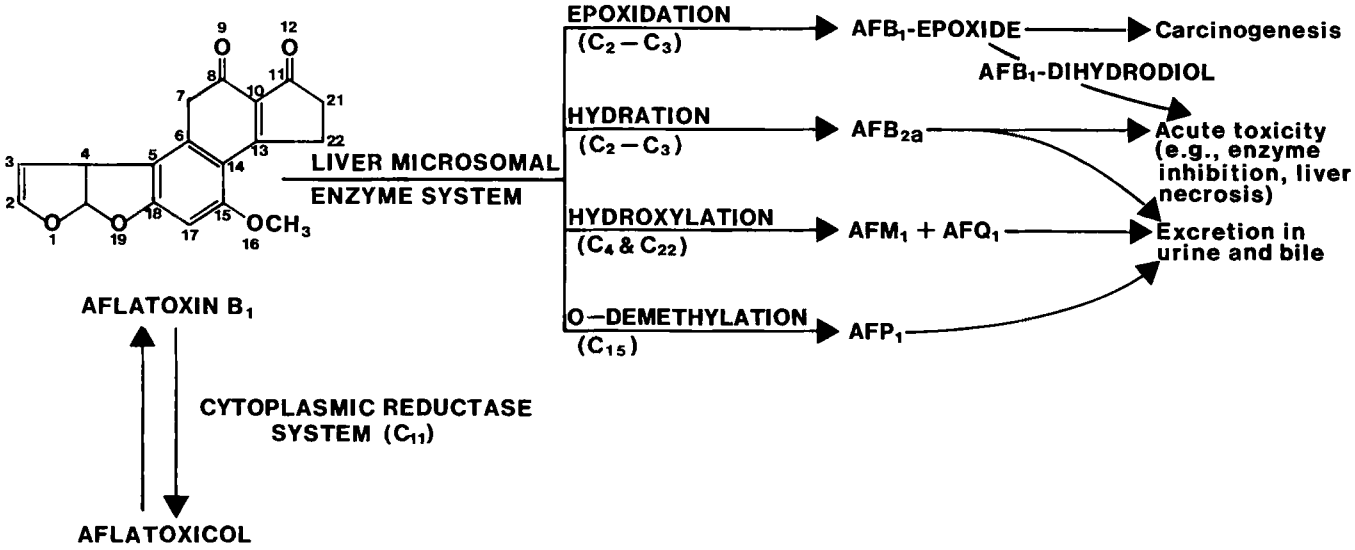

Fig. 1. Metabolic pathways of aflatoxin B_1.

METABOLISM, MECHANISM OF ACTION AND BIOCHEMICAL EFFECTS OF AFLATOXINS

Although quantitative data on absorption of aflatoxins from the gastrointestinal (GI) tract are scarce, their absorption from the GI tract should be complete since very small doses, even in the presence of food, can cause toxicity. After the absorption, highest concentration of the toxin is found in the liver (Mintzlaff et al., 1974). Once in liver, aflatoxin B_1 is metabolized by microsomal enzymes (Figure 1) to different metabolites through hydroxylation, hydration, demethylation and epoxidation. Thus hydroxylation of AFB_1 at C_4 or C_{22} produces, AFM_1 and AFQ_1, respectively. Hydration of the C_2 - C_3 double bond results in the formation of AFB_{2a} which is rapidly formed in certain avian species (Patterson and Roberts, 1970). AFP_1 results from 0-demethylation while the AFB_1 epoxide is formed by epoxidation at the 2,3 double bond. Aflatoxicol is the only metabolite of AFB_1 produced by a soluble cytoplasmic reductase enzyme system.

Aflatoxin B_1 is excreted in urine and feces, and also in milk of lactating animals either unchanged or as various metabolites (Nabney et al., 1967; Allcroft et al., 1968). Only one milk metabolite, namely AFM_1, appears to be the major metabolite of AFB_1 that has shown appreciable oral toxicity (Holzapfel et al., 1966). Its toxicity is considered to be nearly as potent as AFB_1. Even so this metabolite may be detoxified by conjugation with taurocholic and glucuronic acids prior to excretion in the bile or urine (DeIongh et al., 1964; Bassir and Osiyemi, 1967). In this respect, two other metabolites of AFB_1, namely, AFP_1 and AFQ_1 are similar in that they also undergo this type of detoxication (Dalezios et al., 1971; Buchi et al., 1973; Masri et al., 1974). Both of these metabolites are several-fold less toxic than AFB_1. For example, toxicity tests showed that AFP_1 causes some mortality in newborn mice at 150 mg/kg as compared to the LD50 of 9.5 mg/kg for AFB_1 under comparable conditions. It was also found not to be relatively toxic in the chick embryo test system (Stotloff et al., 1972). According to Hsieh et al. (1974), AFQ_1 is approximately 18 times less toxic than AFB_1 in chick embryo test and it is not mutagenic in Ames assay system with or without microsomes (Ames et al., 1973).

It is generally accepted that liver is the target organ for toxic effects of aflatoxin B_1. As a result, metabolism of proteins, carbohydrates and lipids in liver is seriously impaired by AFB_1.

The toxin inhibits RNA polymerase and subsequent protein synthesis at a faster rate in ducks than in rats probably because of faster liver metabolism of AFB_1 in ducks than in rats (Smith, 1965). In day-old chicks, AFB_1 reduces the activity of liver UDP glucose-glycogen transglucosylase resulting in depletion of hepatic glycogen stores (Shankaran et al., 1970). On the other hand, there is lipid accumulation in the liver of chickens and ducklings exposed to aflatoxin (Carnaghan et al., 1966; Shank and Wogan, 1966). With regard to its toxic effects on liver microsomal enzymes, AFB_1 is known to decrease microsomal glucose-6-phosphatase activity (Shankaran et al., 1970) whereas stimulation of microsomal enzyme activity by inducers seems to be unaffected by AFB_1 (Kato et al., 1970). In fact, pretreatment with the toxin actually stimulates its own metabolism in the rat when this is assayed in vitro (Schabort and Steyn, 1969). Since aflatoxin inhibits protein synthesis, it is conceivable why aflatoxin reduces resistance of poultry to infection with Pasteurella multocida, Salmonella spp., Marek disease virus, Coccidia, and Candida albicans (Smith et al., 1969; Hamilton and Harris, 1971). Another effect of aflatoxin is that it causes anti-coagulation of blood. This is probably because AFB_1 inhibits synthesis of factors II and VII involved in prothrombin synthesis and clotting mechanism (Bababunmi and Bassir, 1969).

TOXICOLOGY

Chickens

Susceptibility of chickens to toxic effects of AFB_1 varies with several factors such as breed, strain, age, nutritional status, amount of toxin intake and also the capacity of liver microsomal enzymes to detoxify AFB_1 (Edds, 1973; Veltmann, 1984). Acute toxicity of aflatoxins in chickens may be characterized by hemorrhage in many tissues and liver necrosis with icterus. Although number of field cases of aflatoxicosis in chickens have been diagnosed in various countries, the most severe spontaneous outbreak occurred in North Carolina, in which 50% of a flock of laying hens died within 48 hr of being fed highly toxic maize containing 100 ppm aflatoxin (Hamilton, 1971). The necropsy revealed that liver damage was the most important lesion showing paleness, occasional white pinhead-sized foci and petechial hemorrhages while gallbladder and bile ducts were distended.

Levels of aflatoxin B_1 in moldy feed normally vary from 0 to 10

ppm. At low levels of feed contamination, exposed chickens show, in general, weakness, failure to gain weight with concomitant decline in feed efficiency and egg production (Smith and Hamilton, 1970; Doerr Et al., 1983). Hepatic damage is manifested by enlarged and putty-colored liver, petechial hemorrhages, marked vacuolation of hepatic cells and bile duct proliferation. Feed levels of AFB_1 as low as 250-500 ppb given to New Hampshire chickens have been reported to result in liver damage, decreased hemoglobin, and hypoproteinemia (Brown and Abrams, 1965).

Experimental trials with naturally contaminated feed containing aflatoxin levels ranging from 1-1.5 ppm have caused growth retardation in chickens. Mortality was low but marked hepatic damage was manifested by enlarged and hemorrhagic liver (Carnaghan et al., 1966). Relatively, high dietary levels of aflatoxin B_1 (0-10 ppm) given to Rock type broiler chickens have been reported to cause substantial decrease in weight gain, feed efficiency and hepatic microsomal drug-metabolizing enzymes with concomitant increase in serum glutamic oxalacetic transaminase activity reflecting liver damage (Dalvi and McGowan, 1984; Dalvi and Ademoyero, 1984).

Metabolic alterations caused by aflatoxins in chickens result in elevated lipid levels (Tung et al., 1972; Donaldson et al., 1972), disruptions in hepatic protein synthesis (Tung et al., 1975) which result in several blood coagulation disorders (Doerr et al., 1976; Bababunmi and Bassir, 1982), immunosuppression and decreased plasma amino acid concentrations (Voight et al., 1980).

Turkeys

Acute and chronic toxic effects of aflatoxins, particularly AFB_1, in turkeys are not very different from those in chickens. In the original 1960 outbreak of aflatoxicosis in turkey poults, diffuse necrosis of liver parenchyma, proliferation of the bile duct epithelial cells and frequent hemorrhages were seen (Siller and Ostler, 1961). The prominent cellular changes in liver included swelling and vacuolation of the parenchymal cells, enlargement of the nucleus and in some cases dissolution of nucleolus (Edds, 1973). In an affected flock of turkeys, the symptoms seen were general lassitude, ruffling of feathers and scouring with deaths occurring 3 weeks after the onset of symptoms. Stevens et al. (1960) observed enlargement and congestion of the kidneys as a consistent feature in

turkeys.

Ducklings

Ducklings are considered to be the most susceptible avian species to aflatoxins. Signs of acute aflatoxicosis in ducklings are similar to those seen in chicks and turkey poults, and included anorexia, poor growth rate, ataxia and death in opisthotonus following convulsions. In birds over 3 weeks of age subcutaneous hemorrhages of legs and feet were a characteristic (Asplin and Carnaghan, 1961). Lesions of the liver have been reported to be common in acute and chronic cases of aflatoxicosis in the ducks. The duckling has been recommended as a convenient species for aflatoxin bioassay because of its rapid response to aflatoxin, manifested by marked bile duct hyperplasia 48 to 72 hr after exposure. Prolonged exposure of the duck to low levels of aflatoxins leads to marked nodular hyperplasia of the liver, bile duct proliferation, fibrosis and hepatocellular carcinoma (Carhaghan, 1965).

Carcinogenic Effects of Aflatoxins

One of the serious consequences of aflatoxicosis is the production of hepatocellular tumors. Aflatoxins, mainly AFB_1, have been reported to be carcinogenic in many species with aquatic animals being susceptible and the mouse and hamster resistant (Ueno, 1985). Despite the fact that aflatoxins produce liver tumors in such species as ferret, rainbow trout, pig, guinea pig, monkey, rat and mouse, tumor formation in poultry has not been definitely associated with the ingestion of aflatoxins. Although Carnaghan (1965) was able to observe liver tumors in ducks fed aflatoxin-contaminated ground nut meal, chickens and turkeys have not been shown to develop liver tumors after ingestion of aflatoxins. Since metabolites of aflatoxins are implicated in carcinogenesis and ducks are known to metabolize aflatoxins rapidly, they appear to be prone to aflatoxin-induced carcinoma. For example, there is circumstantial evidence in an outbreak of carcionomas in ducks in India (Kaushik et al., 1972). Furthermore, according to Campbell and Appleby (1966), though not proven experimentally, chickens as young as broilers may develop a wide variety of tumors, some of which may be mycotoxin-related.

PREVENTION

The best way to control aflatoxin formation is to prevent the growth of A. flavus and A. parasiticus on harvested and stored grains and other susceptible commodities. Crops should be harvested at maturity and pre- or post-harvest mechanical damage should be avoided or kept to a minimum since damaged crops favor contamination by toxigenic fungi. Moisture contents of harvested crops should be reduced to a safe level (Knight, 1981). Moisture build-up in the stored grain should be prevented by measures such as regular aeration. There are indications that storage under inert gases may block the elaboration of aflatoxins.

Chemical treatment of stored grain for the prevention of growth of aflatoxin-producing mold has been suggested. Fungicides such as captan may be used for this purpose. However, chronic toxicity of fungicides poses a toxicological problem. Other antifungal agents such as gentian violet and propionic acid have been evaluated and appear to be most promising in the control of aflatoxin-producing fungi. Similarly, benzoic acid has been found to be quite effective against A. flavus (Uriah et al., 1977).

DECONTAMINATION

Although prevention is the best way to reduce aflatoxin contamination of feeds, sometimes it is unavoidable even after taking precautionary measures. Decontamination of such crops/feeds becomes inevitable and can be achieved by physical removal or chemical inactivation of the aflatoxins in the feed. Several methods depending on the nature of contaminated commodities have been suggested for the physical removal of aflatoxins. For example, aflatoxin-contaminated peanuts can be removed by sequential sorting, electronic color sorting or by hand picking (Dickens and Whitaker, 1975). Pneumatic sorting may be applied to lighter, moldy nuts. On the other hand, aflatoxin content of naturally-contaminated cereals can be lowered by milling (Brekke et al., 1975). Yet, the aforementioned methods are unsuitable for the removal of aflatoxins from various types of cereal or nut meals. In those cases, extraction of aflatoxins with aqueous solutions of sodium bicarbonate and calcium chloride has been employed (Sreenivasamurthy et al., 1965). However, the procedure is impracticable in that it

also extracts a large amount of protein. A number of organic solvents have been utilized for the extraction of aflatoxins from various nut meals. For example, a solvent system of acetone, hexane, and water was found to be effective in removing aflatoxins from peanut meal (Rayner and Dollear, 1968). Use of microbes to successfully detoxify some contaminated food products such as milk has also been suggested (Ciegler et al., 1966).

Various chemical agents such as oxidizing agents, acids and bases have shown promise as inactivators of aflatoxins. Of the oxidizing agents which destroy all the aflatoxins, only hydrogen peroxide is considered effective in detoxifying contaminated foods (Sreenivasamurthy et al., 1967). The reduction of aflatoxin levels by inactivation with bases such as sodium and calcium hydroxide is an effective and relatively inexpensive means of aflatoxin removal from contaminated agricultural products (Dollear et al., 1968). Recently, Mora et al. (1982) have suggested the use of a new chloramine disinfectant to detoxify aflatoxin B_1. Another most effective and economically feasible alkaline agent which cleaves aflatoxin molecules at elevated temperature and pressure and decontaminates feeds is ammonia gas. This method is currently being extensively investigated to evaluate the acceptability of the treated product with respect to the nutritional value and toxic hazard. Ammoniated corn fed to white leghorn layer-breeders caused no adverse effects on mortality rates, egg quality or reproduction (Hughes et al., 1978). Similarly, according to Norred and Morrissey (1983), long-term feeding of ammoniated, aflatoxin-contaminated corn resulted in an effective reduction of aflatoxin toxicity in rats.

REDUCTION/REVERSAL OF AFLATOXIN EFFECTS IN POULTRY

Despite the various means of removal of preformed aflatoxons from contaminated agricultural products, complete deconatmination has been a major problem. At present, there does not appear to be any completely successful and practical means, other than avoidance, of removing the preformed aflatoxins from contaminated feed. A possible economical solution would be the development of a non-toxic dietary additive or modification that would make poultry more resistant to the toxic effects of aflatoxins through such mechanisms as decreased absorption from gastrointestinal tract or increased detoxification in liver (Ademoyero and Dalvi, 1983). Adsorption

therapy, in which a toxicant gets bound to an inabsorbable carrier, is one of the most important methods of preventing the absorption of ingested toxicants. Activated charcoal, an effective and non-toxic adsorbent, has been found to be considerably useful in reducing aflatoxin B_1 effects in chickens (Dalvi and McGowan, 1984).

Conjugation of absorbed toxicants or their metabolites with thiols such as reduced glutathione and cysteine is one important detoxification mechanism (Friedman, 1984). Evidence suggests that glutathione or its precursor cysteine alleviates the toxic effects of absorbed aflatoxins in chickens (Ademoyero and Dalvi, 1983) and in goats (Hatch et al., 1977) and regresses hepatocellular carcinoma in rats (Novi, 1981). Another line of approach to enhance detoxification of absorbed aflatoxins is to induce hepatic microsomal enzymes that metabolize aflatoxins, especially AFB_1, to less toxic products. Pretreatment of chickens with enzyme inducers like phenobarbital and fisetin (Dalvi and Ademoyero, 1984) and BHT (Anonymous, 1982) has been found to reduce or reverse the toxic effects of aflatoxins. Other feed additives including selenium and carotenes have also been reported to be of some value in reducing the toxicity of AFB_1 in chickens (Dalvi and Ademoyero, 1984) and in turkeys (Burguera et al., 1983). In addition to the inclusion of above mentioned feed additives, diet modification with high protein (Smith et al., 1971) has also been found to have protective effect against aflatoxins in chickens.

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