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Aflatoxin B_1 in poultry: Toxicology, metabolism and prevention

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ABSTRACT

Aflatoxins (AF) are ubiquitous in corn-based animal feed and causes hepatotoxic and hepatocarcinogenic effects. The most important AF in terms of toxic potency and occurrence is aflatoxin B_1 (AFB_1). Poultry, especially turkeys, are extremely sensitive to the toxic and carcinogenic action of AFB_1, resulting in millions of dollars in annual losses to producers due to reduced growth rate, increased susceptibility to disease, reduced egg production and other adverse effects. The extreme sensitivity of turkeys and other poultry to AFB_1 is associated with efficient hepatic cytochrome P450-mediated bioactivation and deficient detoxification by glutathione S-transferases (GST). Discerning the biochemical and molecular mechanisms of this extreme sensitivity of poultry to AFB_1, will contribute in the development of novel strategies to increase aflatoxin resistance. Since AFB_1 is an unavoidable contaminant of corn-based poultry feed, chemoprevention strategies aimed at reducing AFB_1 toxicity in poultry and in other animals have been the subject of numerous studies. This brief review summarizes many of the key recent findings regarding the action of aflatoxins in poultry.

1. Aflatoxins

Aflatoxins (AF) are the naturally-occurring mycotoxins, produced as secondary metabolites by the fungus *Aspergillus flavus*, *A. parasiticus*, and *A. nominus*. The name "aflatoxin" is derived from the first letter in *Aspergillus*, and the first three letters in *flavus* (Schoental, 1967). Structurally, AFs are difurocoumarin derivatives that fluoresce under ultraviolet light. Depending upon color of the fluorescence, AFs are divided into aflatoxin B_1 and B_2 (AFB_1, AFB_2) for blue, and G_1 and G_2 (AFG_1, AFG_2) for green (Hartley et al., 1963; Dalvi, 1986) (Fig. 1). Aflatoxin M_1 and M_2 (AFM_1, AFM_2), known as milk-AFs, are the metabolites of AFB_1 and AFB_2, respectively (Carnaghan et al., 1963). Other metabolites of AFB_1 are aflatoxin Q_1 (AFQ_1) and aflatoxicol. Aflatoxins are the most intensively researched group of mycotoxins, because of their demonstrated toxic and carcinogenic effects in the susceptible laboratory animals and livestock and their acute toxicological and chronic hepatocarcinogenic effects in humans. Of the known AFs, AFB_1 is the most potent, and is a classified human carcinogen (Wogan et al., 1974; Wong and Hsieh, 1976; Bondy and Pestka, 2000).

2. Toxicity of aflatoxins

The AFB_1 is toxic to a wide range of animal species. AFB_1 is principally an hepatotoxin and hepatocarcinogen (JECFA, 1998), but it causes a myriad of other effects either directly or indirectly associated with this toxicity: immunosuppresion, reduced growth rate, lowered milk and egg production, reduced reproductivity, reduced feed utilization and efficiency and anemia. AFB_1 has been shown to induce hepatocellular carcinoma in many species of animals including fish (rainbow trout, sockeye salmon, and guppy), poultry (turkeys, ducks, and geese), non-human primates (rhesus, cynomolgus, African green, and squirrel monkeys), and rodents (rats, mice, and tree shrews) (Wogan, 1992).

Species susceptibility to various acute toxic manifestations, as measured by TD_{50}, is likewise variable (Gold et al., 1984; Wogan, 1992). While Fisher rats are highly sensitive (TD_{50}: 1.3 mg/kg/body weight/day), Swiss mouse are highly resistant (TD_{50}: >5300 mg/kg/body weight/day). Rhesus and cynomolgus monkeys dosed for an average of 3.3 and 14 years, respectively, yielded a TD_{50} value of 156 and 848 mg/kg/body weight/day, respectively.

A wide variation exists in species susceptibility to AFB_1 hepatocarcinogenesis. Fish and poultry, known to be extremely sensitive to AFB_1, responded to doses as low as 15–30 μg/kg. Rats responded at levels of 15–1000 μg/kg, whereas mice showed no effects to levels as high as 150,000 μg/kg (Wogan, 1992). In rainbow trout, dietary AFB_1 concentrations of 20 μg/kg resulted in a liver tumor incidence of 62% (Bailey et al., 1988).

Non-human primates show a wide variability in AFB_1 susceptibility to hepatic tumors (Adamson, 1989). While squirrel monkeys developed liver cancer when fed AFB_1 at 2000 μg/kg for 13 months, much higher doses were required over a longer period of time to induce low incidence of liver carcinoma in rhesus, cynomolgus and African green monkeys (99–1225 mg/animal administered

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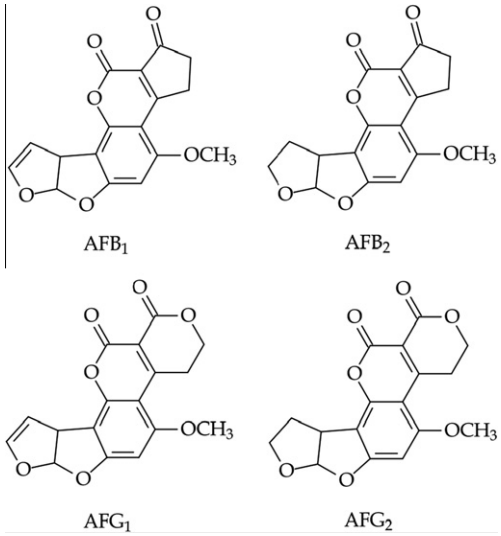

Fig. 1. Chemical structures of aflatoxin B_1, B_2, G_1 and G_2.

p.o. over periods of 48–179 months). However, AFB_1-induced tumors in extrahepatic tissues in the latter species.

In humans, acute aflatoxicosis is manifested by vomiting, abdominal pain, pulmonary edema, coma, convulsions, and death with cerebral edema and fatty involvement of the liver, kidney, and heart (Strosnider et al., 2006). The occurrence of acute aflatoxicosis was evidenced by the severe outbreak in Kenya in 2004 (Probst et al., 2007). Epidemiological studies have consistently demonstrated that AFB_1 is a liver carcinogen in humans (Van Rensburg et al., 1985; Groopman et al., 1988). Studies conducted in Swaziland and Guangxi, China has linked AFB_1 exposure to development of liver cancer in humans (Peers et al., 1987; Yeh et al., 1989). The International Agency for Research on Cancer has concluded that there is sufficient evidence for the carcinogenicity of AFB_1 in humans and hence placed this mycotoxin under group I.

Aflatoxin B_1 is a "pro-carcinogen" in that enzymatic bioactivation is a prerequisite for carcinogenic (and toxic) activity (Garner et al., 1972). Accordingly, elucidation of the mechanisms of AFB_1 metabolism has been the focus of intense research over the years. AFB_1 is metabolized by hepatic microsomal cytochrome P450s (P450) to the reactive, electrophilic *exo*-AFB_1-8,9-epoxide (AFBO) which binds to DNA and other critical cellular macromolecules (Ball and Coulombe, 1991; Wogan, 1992; Coulombe, 1993; Gallagher et al., 1996; Guengerich et al., 1996). The AFBO is highly unstable, and it reacts with the DNA to form N^7 guanine adducts by intercalation of AFBO between base pairs (Iyer et al., 1994).

There are a number of urinary and serum biomarkers that have been validated to accurately predict AFB_1 cancer risk in humans. These include urinary aflatoxin-N^7-guanine and AFM_1 (Gan et al., 1988; Groopman et al., 1992; Groopman and Kensler, 1999). Urinary excretion of the mercapturic acid (AFB-NAC), a product of glutathione (GSH) adduction of AFB_1 mediated by glutathione-S-transferases (GSTs), has also been used in field studies (Wang et al., 1999). Serum AFB-albumin adducts, which are positively associated with hepatocellular carcinoma in humans (Wang et al., 1996) have found wide use in epidemiologic studies (Wild and Turner, 2001). Analysis of serum adducts indicates a positive correlation between dietary AFB_1 exposure and serum AFB-albumin adducts (Gan et al., 1988; Wild et al., 1992).

3. Aflatoxin B_1 toxicity in poultry

Poultry, especially turkeys are extremely sensitive to the toxic effects of AFB_1 (Carnaghan et al., 1966; Arafa et al., 1981; Giambrone et al., 1985; Huff et al., 1986; Kubena et al., 1995; Klein et al., 2000). Extreme sensitivity of turkeys to AFB_1 was first and graphically demonstrated by association with 'Turkey X Disease' which caused widespread deaths of turkeys and other poultry throughout Europe in the 1960s (Stevens et al., 1960). The disease was shown to be caused by AFB_1 contaminated feed (Smith, 1960). It was later reported that the contamination with AFs came from Brazilian peanut meal (Blount, 1961). Among different poultry species, turkeys are shown to be the most susceptible to AFs, quails are intermediate, while chickens are considered relatively resistant (Arafa et al., 1981; Lozano and Diaz, 2006). Although direct comparisons have not been conducted, wild turkeys appear to be less susceptible to AFB_1 than their commercial counterparts (Quist et al., 2000).

Aflatoxin at 0.7 mg/kg reduced the growth rate of turkey poults, but had no effect in quails and chickens (Arafa et al., 1981). A diet containing 400 mg/kg AFB_1 severely affected body and relative liver weights in turkeys, while chickens showed no effect at this dietary concentration (Leeson et al., 1995). A study examining the effects of AFB_1 on the development of liver lesions in poults, reports ducks as more susceptible than turkeys and chickens (Coker, 1979). In that study, ducks developed hepatic lesions by dietary exposure to 30 μg/kg, turkeys at 300 μg/kg, while chickens responded to 500 μg/kg. Another study evaluated the effects of AFB_1 on the development of cytopathology in the tracheal culture in day-old turkeys, Japanese quails, chicken and ducks (Colwell et al., 1973). While cultures derived from ducks developed pathology at 6 μg/kg AFB_1, the levels needed for equivalent pathology were as high as 100 μg/kg for those from chickens. Tracheal cultures from turkeys and quails responded to 28 and 47 μg/kg of AFB_1, respectively.

4. Economic Impacts to the poultry industry due to aflatoxins

Turkeys are an important international food commodity. The United States accounts for roughly one-half of the world's turkey production at approximately 7.30 billion pounds live weight, with an estimated value of nearly US$ 3 billion (National Agricultural Statistics Service, USDA). The per capita consumption of turkeys in the United States is approximately 18 pounds, and turkeys are now the fourth major food and protein source, behind chicken, beef and pork, respectively (National Turkey Federation).

Aflatoxins result in economic losses to poultry industry from reductions in growth rate, hatchability, feed efficiency and immunity towards diseases (Richard et al., 1986; Coulombe, 1993). According to a report by Council for Agricultural Science and Technology, losses due to AFs to the United States poultry industry exceeded $143 million annually (CAST, 1989). A recent study reported annual crop losses of $932 million due to mycotoxin contamination and additional losses of $466 million in efforts to prevent or reduce contamination (CAST, 2003). Although AFs are found in most of the feed ingredients; corn, peanut meal, cottonseed meal, and sorghum appears to be at greatest risk for introducing AFs in turkey diets (Pons and Goldblatt, 1965; Brekke et al., 1977; Winn and Lane, 1978; Hill et al., 1983). Crops contaminated with AFs are a worldwide problem and approximately 25% of world's food supply is contaminated with mycotoxins annually (CAST, 1989). Conditions that favor contamination by mycotoxins include excessive moisture both in field and post harvest storage, high humidity, temperature extremes, drought stress, and insect damage to crops (Coulombe, 1993). Aflatoxins are deleterious to poultry and their contamination in feed is practically unavoidable (Coulombe et al., 2005). The United States Food and Drug Administration regulates the amount of AFB_1 in poultry feed. Current action