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Aflatoxins in Poultry

Introduction

Aflatoxins (AF) are mycotoxins that are produced by various *Aspergillus* species including *A. flavus, A.parasiticus* and *A. nominus*. As secondary metabolites of these fungi, AF may contaminate a variety of food and feedstuffs, especially corn, peanuts and cottonseed. Chemically, AF are difuranocoumarin compounds and include aflatoxin B_1, B_2, G_1, G_2, M_1 and M_2 depending on their structures (Figure 1). Aflatoxin M_1 and M_2, however, mainly occur in milk (small quantities of AFM_1 have been reported in eggs) as metabolites of AFB_1 and AFB_2, respectively.

Among the known AF, AFB_1 is most commonly encountered and considered the most toxic (classified as a human carcinogen; Yunus et al, 2011). AF have been given considerable attention because of their demonstrated carcinogenic potential and hepatotoxic effects in both humans and animals. In animals, adverse effects of AF also include reduction in growth rate and feed efficiency, decreased egg production and hatchability, and increased susceptibility to disease. In addition, residues of AF from animals can appear in edible animal products for human consumption, which raises public health concerns.

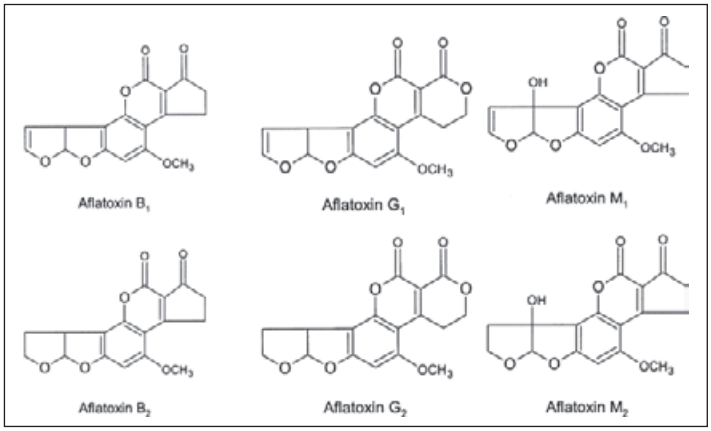

Figure 1. Chemical structure of AFB_1, AFB_2, AFM_1, AFM_2, AFG_1 and AFG_2 (Zain, 2011)

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The discovery and isolation of AF can be traced back to the mysterious Turkey-X disease of 1960, which resulted in the loss of several thousand turkey poults in the United Kingdom. AF contamination is still a threat to the poultry industry and results in substantial economic losses to producers because of often sub-lethal, but toxic, effects. The U.S. Food and Drug Administration (FDA) has established guidelines for the maximum toxin level that can be safely fed to poultry (Table 1).

Table 1. FDA's action levels for aflatoxin in poultry feed

Class of Animal	Feed	Maximum Aflatoxin Level (mg/kg)[1]	(ppb)
Immature poultry	Corn & peanut products	0.02	20
Mature poultry	Corn & peanut products	0.1	100
Poultry	Cottonseed meal	0.3	300
All animals	Other feeds	0.02	20

[1]Concentrations are reported as either ppm (parts per million) or ppb (parts per billion). Because of differences in international interpretation, metric concentrations are utilized in this publication.

1 mg/kg = 1 ppm = 1000 ppb.

These regulatory limits have implications for international trade in grain crops and, in some instances, can result in a barrier for the export or import of commodities from different parts of the world. Importantly, FDA generally does not permit corn containing AF to be blended with uncontaminated corn to reduce the AF content of the resulting mixture to levels acceptable for use as human food or animal feed. However, on occasion FDA has relaxed its "no-blending" policy in response to widespread outbreaks of AF (as occurred in 1988) or in response to state-specific requests to address local outbreaks (as was allowed in Indiana during the 2012 harvest).

Poultry Productivity Upon AF Exposure

There is general agreement that dietary AF reduces weight gain and feed intake, and worsens feed efficiency. The response of animals to AF-contaminated feed depends on the AF concentration, animal species, and age and sex. Previous research indicated that the reduced growth rate because of AF ingestion in the diet is primarily due to the reduction in feed intake. If the feed is contaminated by multiple mycotoxins at the same time, AF can interact with other mycotoxins, such as ochratoxin A and T-2 toxin, to produce more severe effects on broiler performance than individual mycotoxins.

Research from the past few decades has illustrated the negative effects of AF on poultry performance. However, little attention has been paid to the chronic ingestion of small amounts (≤1 mg/kg) of AF. A summary of broiler performance response to low dietary concentrations of AF is shown in Table 2. From the available information, birds fed diets that contain AF as low as 0.3 mg/kg start to show reductions in growth rate, feed intake and a worsened feed efficiency. Based on the meta-analysis of 98 papers from 1980-2009, Andretta et al (2011) concluded that an average AF concentration of 0.95 mg/kg reduced both feed intake and daily weight gain by 11 percent, and worsened feed conversion by 6 percent.

Table 2. Summary of the response of broiler performance to dietary aflatoxin

AF (mg/kg)	Changes in performance			AF source	Duration	Reference
	ADG (g/d)	ADFI (g/d)	Feed/ gain (g/g)			
0.3	-11.80	-14.26	+0.07	Cultured material	5 wks	Raju and Devegowda, 2000
0.4	-5.48	---	---	Unknown origin	7 wks	Sodhi et al, 1996
0.5	-1.35	-0.16	+0.11	Cultured material	7 wks	Verma et al, 2004
0.5	-4.6	No effect	+0.18	Cultured material	5 wks	Manafi et al, 2012
0.675	-2.295	---	---	Cultured material	7 wks	Doerr et al, 1983
0.81	-0.48	-0.16	+0.11	Naturally contaminated	5 wks	Giambrone et al, 1985
1	-5.12	-5.16	+0.26	Cultured material	7 wks	Verma et al, 2004
1.14	-2.6	0.19	+0.13	Naturally contaminated	6 wks	Shi et al, 2009
2	-3.7	---	---	Cultured material	3 wks	Basmacioglu et al, 2005
4	-9.33	-10.38	+0.04	Purified AFB1	3 wks	Ledoux et al, 1999
5	-8.5	---	+0.35	Purified AFB1	3.4 wks	Randall and Bird, 1979

AF also can affect laying hens and lead to reduced egg production, poor egg quality and increased mortality of challenged hens. AFB_1 adversely influences egg quality by decreasing shell thickness, egg weight and egg energy deposition. The negative impacts of AF on laying hens can be induced when feed contains 1-2 mg/kg (Azzam and Gabal, 1998; Verma et al, 2007). In addition, AF in laying hen feed can result in an AF residue in the eggs (feed to egg AFB_1 transmission ratio was approximately 5000:1); therefore it is very important to control AF concentrations in feeds for laying hens (Oliveira et al, 2000).

Liver as a Key Player of AF Toxicity and Sensitivity Within Poultry Species

Following absorption of AF in the upper part of the small intestine (80-90 percent of what is eaten is absorbed), AF undergoes an extensive transformation into metabolites in the liver. In fact, AF are not toxic *per se*, but require metabolic conversion by hepatic enzymes (the cytochrome P450 family) to the metabolically active metabolite *exo*-AFB1-8, 9-epoxyde (AFBO) to exert its toxicity. This metabolically active form of AF can bind with particular cellular compounds (proteins, DNA and

RNA) to influence normal cellular activities, and is considered the active form responsible for the carcinogenicity and mutagenicity of AF.

The extreme sensitivity of poultry species to AF is associated with their livers converting efficiently AF to the metabolically active AFBO. This susceptibility has, however, been shown to differ among poultry species. Ducks are reported to be the most susceptible poultry species to AF, followed by turkeys, broilers and laying hens, with ducks being approximately 200 times more sensitive than chicks, especially for acute hepatotoxic effects (Diaz and Murcia, 2011; Bintvihok, 2001). Ducklings exhibit 100 percent mortality at 1 mg/kg AF (Muller et al, 1970), and ducks are the only poultry species that develop hepatocellular carcinoma upon AFB_1 exposure (Diaz and Murcia, 2011). One explanation for the high sensitivity of ducks to AFB_1 could be that the enzymes (cytochrome P450 family) responsible for bioactivation of AFB_1 show a higher activity than in chickens, turkeys or quails (Diaz and Murcia, 2011). In addition, the lower tolerance of ducks also could be explained by a lower activity of hepatic enzymes responsible for cellular detoxification and excretion of a variety of toxic substances.

It is interesting to note that many authors who reviewed studies conducted prior to the 1980s considered 1 mg AFB_1/kg as not having any negative effects on broiler performance. In contrast, recent literature reported adverse effects at concentrations as low as 0.02 mg/kg. A plausible explanation of these differences between earlier and more recent reports could be that modern broilers have more efficient nutrient conversion demanding faster hepatic metabolism, which in turn results in a higher metabolism of AFB_1 (Yunus et al, 2011).

Immune and Metabolic Disorders Upon AF Exposure

AF acts as an inhibitor of protein synthesis and, subsequently, dividing cells and tissues with a high protein turnover such as that found in the liver, immune system or gut epithelium, which is most susceptible to the toxic effects of AF. In this respect, exposure to AF has been demonstrated to suppress the immune response in poultry. AF can repress the development of the thymus gland or influence the relative weight of the bursa of Fabricius, which may result in serious deficiencies in both cellular and antibody responsiveness of the chicken immune system (Celik et al, 2000). Inhibition of macrophage functions, T lymphocyte activity or cytokine expression by AF results in vaccine failure or pathogen persistence, as exemplified in many studies by reduced immunoglobulin production (Verma et al, 2004; Yunus et al, 2011).

Recent epidemiological data indicates a high correlation between outbreaks of Newcastle disease and AF contamination of broiler rations (Yunus et al, 2011). In general, the dose of AFB_1 needed to affect the immune system is considered less than the dose required to elicit a reduction in bird performance. The threshold dose of AFB_1 is reported to be approximately 0.4 and 1 mg/kg for the negative effects on cell mediated and humoral immunity, respectively, in broilers (Yunus et al, 2011). Therefore, chronic consumption of feed contaminated with low AF content may pose a serious risk to animal health, increasing susceptibility to infections or reducing vaccination efficacy.

The gastrointestinal tract is the first organ coming into contact with mycotoxins of dietary origin, and should be expected to be affected by AFB_1 with greater potency as compared to other organs. However, this aspect of aflatoxicosis is the area most often neglected in mycotoxin research. Limited data suggest that the absorptive surface of the small intestine deteriorates during chronic exposure to low levels of AFB_1.

Some reports have concluded that enzyme activities are modulated following AF consumption. An increased release of enzymes from the pancreas to the intestinal tract might be a consequence of pancreatic damage (Grenier and Applegate, 2012). Very low doses of AFB_1 (0.02 and 0.04 mg/kg) have reduced the apparent digestibility of crude protein by 8-13 percent in ducks. Similarly, it has been suggested that dietary AF increases the amino acid requirements, and it appears to negatively impact ducks more than chickens (Grenier and Applegate, 2012). In addition, AF has been shown to reduce energy utilization and, in combination with ochratoxin A, had a more pronounced affect on metabolizable energy than when either was fed alone. This reduction occurred through a significant increase in the maintenance energy requirement of the hen (Verma et al, 2007). Moreover, there is a loss of energy availability in the feed because of the feeding of moldy corn containing mycotoxins.

Conclusions

Generally, 0.95 mg/kg AF in the diet reduces weight gain by 11 percent because of, in part, reduced feed intake and metabolic inefficiencies from liver and GIT damage. The metabolic pathways of AFB_1 in the liver are very complicated, and not all species go through the same reactions. However, duck producers, in particular, need to pay particular attention because of the duck's low resistance to AF. While exact dosages that birds will receive cannot be predicted, feed ingredient testing strategies in times of higher occurrence can help. Additionally, sequestration/adsorption of AF by feed additives (such as clay and mineral zeolites) can lessen the severity of these impacts. For further information on these products and their efficacy, refer to Purdue Extension publication AS-614-W, *Reducing the Impact of Aflatoxins in Livestock and Poultry* (http://www.extension.purdue.edu/extmedia/AS/AS-614-W.pdf).

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