Chapter

Aflatoxin Occurrence, Detection, and Novel Strategies to Reduce Toxicity in Poultry Species

Surya Kanta Mishra and Bijaya Kumar Swain

Abstract

Aflatoxins (AF) are the commonly occurring mycotoxins 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. Among the many known AFs, AFB1 is the most commonly encountered and the most toxic. In poultry, adverse effects of AF include reduction in growth rate and feed efficiency, decreased egg production and hatchability along with increased susceptibility to diseases, besides residues in food chains. Many rapid screening methods for detecting aflatoxin are available currently, namely: thin layer chromatography (TLC), HPTLC, HPLC, enzyme-linked immunosorbent assay (ELISA), monoclonal antibody kits, and affinity column chromatography, making the detection of AF precise. For field application, rapid assay kits, e.g., Aflatest of Vicam and Afla-2-cup of Romers Labs, are currently available. The most novel ways to counteract aflatoxin already accumulated in the feed could be by getting them bound to inert compounds before absorption from host's intestine. Among various classes of poultry, ducks followed by turkeys form the two most vulnerable poultry species, among others. Considering the inherently high genetic variation between duck breeds for AFB susceptibility, a genetic selection program to improve AFB resistance can be a long-term option. Further epigenetic sensitization of the AFB-susceptible poultries through mild AFB exposures is getting reported as an emerging genetic approach to counter AFB susceptibilities. The chapter discusses most of these, in greater detail.

Keywords: aflatoxin, detection method, occurrence, detoxification, poultry, susceptibility

1. Introduction

An outbreak of Turkey-X disease in the United Kingdom in 1960s following the ingestion of poultry feed containing Brazilian ground nut cake led to the discovery of a group of compounds, which are now known as aflatoxins (AFs). Chemical and microbiological investigations soon revealed that the toxic effects produced by Brazilian ground nut cake had resulted from the presence of four secondary metabolites of the mold *Aspergillus flavus* in the diet [1].

Aflatoxins (AFs) are difuranocoumarins mainly produced by two *Aspergillous* species, namely *Aspergillous flavus* and *Aspergillousparasiticus* [2]. According to their chemical structures, there are two main categories of AFs; the first category being difuranocoumaro-cyclopetene group and includes aflatoxins B1, B2 (AFB1, AFB2) while the second category is formed by the AFG1 and AFG2. The nomenclature of AFB1 and AFB2 is derived from the blue fluorescent color produced and visualized under UV light while AFG1 and AFG2 produce green fluorescent color [3, 4]. Among all the discovered mycotoxins, aflatoxins form the most elaborately researched group, because of their toxicological and hepatocarcinogenic effect in various susceptible animals. The toxigenicity among four AF compounds has been rated in order such as: B1 > B2 > G1 > G2. Chemically, AFs are polycyclic unsaturated compounds consisting of a coumarin nucleus flanked by a highly reactive bifuran system on one side and either a pentanone or a six member lactone on the other side. The toxic nature of AFs is due to its chemical structure. The lactone ring undergoes epoxidation to produce AFB1 2-3 epoxide, which accounts for its toxic properties. Any alteration in opening of lactone ring or saturation of double bond associated with lactone ring causes reduction in the toxicity [5]. Consumption of AF contaminated agricultural stuffs thus becomes the main route of exposure in poultry. Major adverse effects of AFs are loss of appetite, decreased feed intake, poor feed utilization, immunosuppresion, decreased egg production, and increased mortality in poultry [6–8] and additionally, the suppression of immune system [9, 10]. Immunosuppressive, hepatotoxic haemorrhage [11], carcinogenic, mutagenic, growth inhibitory [12], and teratogenic [13] effects can be detected according to animal species, sex, age and aflatoxin type, exposure dose and period. The median lethal dose (LD_{50}) of AFB1 is estimated to be between 0.3 and 18 mg/kg according to the route of administration, species of animal, age, sex, and health condition. Poultry are usually more susceptible to AFs than mammals. Within poultry, ducks are most susceptible species of all, followed by the turkey poults and thereafter, the chickens. Young animals are more susceptible to AFs than matured animals. Nutritional deficiencies, especially protein and vitamin E, increase the susceptibility to AFs [14]. Decrease in nutrient absorption in broilers fed AFB1-contaminated diet is because of the effect of toxin on systemic metabolism and not an effect on digestive functionality [15, 16].

Physical, chemical, and biological methods are essential to counteract the levels of contamination of AF, already accumulated, in foods and feeds. The cost involved and reduction in nutritive value of feed are some of the constraints that limit the use of such procedures during the feed preparation. Various studies indicate that it is practically not possible to totally eliminate the molds and their toxins from the feed. Therefore, there is need to use suitable agents that are capable of binding the toxins selectively in the gut, thus limiting their bioavailability to the consumer. Further, presence of toxic residues in poultry products (egg, meat), which enters in to the food chain, may pose potential risk by their hazardous effects on the health of human beings [17]. An approach to the aflatoxin contamination problem has been to use non-nutritive and inert adsorbents in the diet to bind AF and reduce the absorption of AF from the gastrointestinal tract. Use of adsorbents such as zeolites and alluminosillicates has proven successful, but their possible interaction with feed nutrients is a cause of concern [18, 19]. Therefore, the occurrence of AF, its detection procedures in different feedstuffs and different strategies to ameliorate its effect on the performance of poultry, and the reduction of their residues in food for food safety are discussed in detail below.

2. Occurrence

Aflatoxins were first identified in early 1960s and since then have been the most studied mycotoxins. Aflatoxins (AFs) are the most commonly occurring mycotoxins that are heterocyclic compounds produced as secondary metabolites mainly by various *Aspergillus* species including *A. flavus, A.parasiticus,* and *A. nominus* [20]. The biosynthesis of AFs consists of 18 enzymatic steps with at least 25 genes responsible for producing the enzymes and regulating the biosynthetic process [21, 22]. These mycotoxins are mainly found in agricultural products in tropical and subtropical regions [23–25]. Almost all agricultural commodities will support the growth of aflatoxin-producing fungi *A. flavus, A. parasiticus*. Formation of AF can occur during the pre and post-harvest stages of food production as long as a suitable environment for mold growth is available. Optimal conditions for AF production are a water activity in excess of 0.85 (85% RH) and a temperature of 27°C, conditions that are frequently encountered in Mediterranean region. Different crops vary in their ability to support fungal colonization because of differences in the chemical composition of each commodity. The incidence and degree of AF contamination vary with seasonal and geographical factors and also with the conditions under which the crop is grown, harvested, stored, and transported [26]. Factors affecting the production and occurrence of mycotoxins in crops and the level of contamination in feed and food entail climatic conditions such as temperature, relative humidity, and agricultural operations such as usage of fungicides. Other factors include: drying, processing, handling, packaging, storage, and transport environment. Insects play an important role in contaminating the agricultural commodities through physical damage of the grains and mechanical transmission of the microorganisms [27–30]. As such, most of the cereal grains, oil seeds, and tree nuts are susceptible to fungal invasion and consequently formations of mycotoxin aflatoxin. Agricultural products such as cereal grains and forages can be polluted during pre-harvest [field period, harvest, and post-harvest (storage and transportation period)]. Maize and other grains used in poultry feed could also be infected by pathogenic molds and thereby produce aflatoxins, even when they may be destroyed at different rates during industrial processing [2, 14, 31, 32]. The fungal species can invade foods and feedstuffs depending upon the geographical and climatic conditions of a particular region. Aflatoxins are mostly expected in tropical areas where climatic conditions and storage practices are favorable to fungal growth and toxin production, whereas other mycotoxins such as ochratoxins and fumonisins are detected in moderate, subtropical and tropical locations, with zearalenone and trichothecenes forming the worldwide mycotoxins [33, 34]. Unfortunately, the food and feed contamination by AFs is a persistent problem worldwide. The outbreaks due to AFs are more prone in tropical and subtropical areas, with a few in temperate regions. Further, the Mediterranean zones have become prone to AFs contamination due to shifting in traditional occurrence areas of AFs because of climate change, namely increase in average temperature, CO_2 levels, and rainfall pattern [35]. This has led to an increased occurrence of AFs worldwide, due to increase in contamination of crops.

Aflatoxins are often present in feedstuffs and cause some adverse effects, which can range from: vomiting, weight loss, and acute necrosis of parenchyma cells to various types of carcinoma and immunosuppression in large animals, pets, and poultry birds [36, 37]. Aflatoxin B1 (AFB1), among the four major types of AFs, is the most toxic and potent carcinogen in humans and animals [38]. AFB1 causes series of pathophysiological changes in an organism such as lower growth rate, malnutrition,

silenced immune response, and disturbed gastrointestinal tract. Also, AFB1 can induce various histopathological manifestations of hepatocytes such as proliferation of the bile duct, centrilobular necrosis and fatty degeneration of the hepatocytes, and hematoma [29, 39–41]. AFB1 is already reported to induce hepatocellular carcinoma in many species of animals including fishes (rainbow trout, sock eye 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) [36, 42]. In poultry, AFB1 mainly affects the liver, kidney, immune organs (spleen, bursa of fabricius, and thymus), and gastrointestinal system. Poultry industry, factually, is one of the largest, most organized, fastest-growing, and vibrant segments of agro-industries, generating direct and indirect employment and income for millions of people, in developed and developing countries [43–45]. According to an estimate by the Food and Agriculture Organization (FAO), 25% of the world's food crops are affected by mycotoxins, and the rate of mycotoxin contamination is likely to increase in line with the trend seen in preceding years [46–49]. A worldwide mycotoxin survey in 2013 revealed that 81% of around 3000 grain and feed samples analyzed had at least one mycotoxin, which was way higher than the 10-year average (from 2004 to 2013) of 76%, in a total of 25,944 samples. The most notorious mycotoxins, thus, are aflatoxins (Afs), which often result in low performance in poultry, decreased quality of egg and meat production, and then, cause significant economic losses [50–52]. In broilers, aflatoxins drastically affect almost all valuable production factors including weight gain, feed intake, and feed conversion ratio (FCR) and induce immunosuppression, which is directly related to reduced effectiveness of vaccination programs, increased risk of infectious diseases, and high mortality. In layers, aflatoxins cause the decrease in egg production, egg size, and egg quality.

Included in the text, is a tabular presentation of various feed materials/grains with mention of their aflatoxin contamination ranges along with incidence rates (**Table 1**).

Food materials	Class of aflatoxin	Incidence rate (sample size)	Detection range	Country	References
Peanut	AFB1	57 (49)	LOD to 193 μg/kg	Algeria	[53]
Maize	Aflatoxin Total	40 (270)	—	Argentina	[54]
Maize	AFB1, AFG1		0.5–49.9 μg/kg	Brazil	[55]
Peanut	AFTotal	10 (119)	0.3–100 μg/kg	Brazil	[56]
Maize	AFB1	2.3 (44)	0–148.4 μg/kg	China	[57]
Wheat and Wheat crackers	AFB1	5.6 (178)	0.03–0.12 μg/kg	China	[58]
Peanuts	AF Total	0.15 (2494)	0.06–1602.5 μg/kg	China	[59]
Maize	AFB1, AFB2, AFG1	15, 15, 5 (20)	1.9–458.2 μg/kg	Columbia	[60]
Rice	AFB1	12.5 (24)	100–200 μg/kg	Egypt	[61]
Wheat	AFB1	33.33 (36)	<LOD to 49.79 μg/kg	Egypt	[62]
Maize	AFB1, AFB2	24.6 (61)	0.02–0.19 μg/kg	Egypt	[63]
Maize	AF Total	100 (150)	20–91.04 μg/kg	Ethiopia	[64]
Sorghum	AF Total	100 (90)	<LOD to 33.10 μg/kg	Ethiopia	[65]

Food materials	Class of aflatoxin	Incidence rate (sample size)	Detection range	Country	References
Peanut and peanut cake	AF Total	32 (160 peanut) 68 (50 peanut cake)	<LOD to 2368 μg/kg <20–158 μg/kg	Ethiopia	[66]
Sesame seeds	AFB1	77.6 (30)	LOD to 14.49 μg/kg	Greece	[67]
Maize	AF Total	37.7 (326)	<LOD to 341 μg/kg	Ghana	[68]
Sorghum	AFB1	71.42 (15)	0.005–0.02 μg/kg	India	[69]
Rice	AF Total	2.3 (87)	21.581–22.989 μg/kg	India	[70]
Rice	AFB1	100 (40)	0.29–2.9 μg/kg	Iran	[71]
Maize	AF Total	75 (140)	—	Italy	[72]
Sorghum	AFB1, AFB2, AFG1, AFG2	10.81, 5.41, 18.92, 32.43 (37)	—	Kenya	[73]
Sorghum unit	AFB1, AFB2, AFG1	44, 9, 17 (45)	0.61–28.3, 0.14–2.35, 0.39–6.95 μg/kg	Namibia	[74]
Sorghum	AFB1	28.6 (146)	0.96–21.74 μg/kg	Nigeria	[75]
Rice	AF Total	36.9 (38)	00–20.2 μg/kg	Nigeria	[76]
Rice	AF Total	50 (72)	0–40 μg/kg	Pakistan	[77]
Maize	AF Total	64.6 (82)	1–17 μg/kg	Peru	[78]
Maize	AF Total	48.2 (56)	LOD to 9.14 μg/kg	Serbia	[79]
Maize	AFB1, AFB2	1 (507)	5.2 μg/kg	South Korea	[80]
Peanut	AF Total	25 (1089)	LOD to 432 μg/kg	Taiwan	[81]
Maize	AF Total	4 (1055)	7.96–163.62 μg/kg	Turkey	[82]
Wheat	AF Total	2 (141)	0.21–0.44 μg/kg	Turkey	[83]
Peanut	AF Total	84 (102)	0.2–2177.2 μg/kg	Turkey	[84]
Sorghum	AFB1	0.7 (275)	1–14 μg/kg	Uruguay	[75]

Table 1.
Surveys of food and agricultural products contaminated with aflatoxin in different locations.

3. AF-susceptible poultry species, inter and intra-species variations: current research

3.1 Genetic variation within various poultry for susceptibility to aflatoxicosis

It is now well established that susceptibility of a poultry species to aflatoxicosis is subject to variation due to underlying genetic makeup of the host. This would mean that there already exists an inter-species variation across current range of domesticated poultry species, with respect to their threshold of clinical tolerance. The global literature is now replete with multiple reports, citing how common poultry ducklings, goslings, and turkey poults are viewed as the most susceptible in contrast to female rats (too resistant), in terms of host-to-host comparison for aflatoxin metabolism within system [85–87].

Taking leads from such literature, further reports from around the globe have well indicated a definite variability among species for degree of susceptibility across species; across breeds and genetic lines. It is well determined that ducklings and turkey

poultry turn out to be the most sensitive species to aflatoxins. Next are the species of goslings, quails, and pheasants, which display intermediate sensitivity, in that scale. Hearteningly, using the same yardstick, the chickens appear to be the most resistant [88] to lethality, from aflatoxin-contaminated feeds. Earlier researchers [89] have demonstrated that the chicks can tolerate up to 3 ppm AFB in the diet without showing any significant adverse effects. A separate study found out that chickens are not only highly resistant to adverse effects of AFB1, but there could still be some modest enhancement in the body weight of chickens, when exposed to aflatoxin-contaminated diets, leading to a finding that was characterized as an hormetic-type dose-response relationship [90]. The most specific and relevant study for inter-species susceptibility evaluation by [91], who have concluded that "the susceptibility-variation among five distinct species of poultry, lied in the order of ducklings > turkey poults > goslings > pheasant chicks > chickens" in decreasing order of susceptibility, among commercial poultry. This study further documented that ducklings were 5–15 times more sensitive to aflatoxin's effects than those of laying hens, with respect to productivity outputs. Further, when the laying hen strains were compared, inter se, certain strains of hens turned out to be nearly thrice more sensitive than other strains [92].

3.2 Aflatoxicosis in ducks

As the ducks appear to be the most vulnerable species to the aflatoxicosis effects, among entire domesticated poultry, a renewed emphasis is currently on to study the whole spectrum of toxicological effects resulted in the ducks, which impact the productivity in ducks.

Way back in mid-twentieth century, when the aflatoxicosis was being described in literature, just as "*Turkey-X disease*," the report of previous research workers [93] documented that toxicological impacts from aflatoxicosis (in ducks) resulted in inappetance, abnormal vocalizations, reduced growth, besides feather picking tendencies, purple discoloration of legs and feet resulting in lameness in ducklings upon feeding with AF-contaminated diets. The typical symptoms of ducklings included: ataxia, convulsions, and opisthotonos, preceding death from aflatoxicosis. Lameness, either unilateral or bilateral, as an outcome of long-term feeding of AFB1-spiked diets (@ 200 ppb for 6 weeks) to Pekin ducks was also reported [94] resulting in near condemnation of the survivor ducks as meat animals, owing to obvious reasons. The reports of Indian labs (author's own lab at ICAR-DPR) have also shown that recurrent presence of naturally arisen AFB1 (in 30–50 ppb ranges) in Pekin ducks has largely been the reason behind huge condemnation of the aflatoxicosis survivors, which not only gave rise to carcass degradation, but also affected the usual fleshing of meat-type Pekin ducks at marketable ages, say by 6–8 weeks latest [95].

Huge genetic variation with respect to morbidity and mortality of ducks on production and fitness, even at an organized farm, has been reported between breeds of domesticated ducks, in conditions of natural aflatoxicosis [96], where duck's fertility (FRT), hatchability on total set (HTES) besides survival of adult layers were significantly affected during the laying period (20–72 weeks of age), whenever the AFB1 levels breached the 10 ppb levels in the naturally stored diets. The between-breed variation with respected to survival and production drops was settled as: The susceptibility to aflatoxins was in the order: Pekins > natives > Khaki Campbells. The authors concluded that: there remained a need for an anti-toxin duck-raising strategy, which can be based on genetics and climatic factors, including a vigilant feeding and healthcare regime.

The postmortem lesions in ducks have also been detailed by many authors to detail the organ specific changes accumulated to duckling. Many authors have reported hepatitis and nephritis with enlarged and pale kidneys. As regards the chronic effects of AFB, ascitis and hydropericardium have been reported, which were accompanied by shrunken firm nodular liver; distention of the gall bladder and hemorrhages, distended abdomen due to liver tumors and secondary ascites [97, 98].

Various microscopic lesions in the liver have been reported from AFB1 by above authors, which included fatty change in hepatocytes; proliferation of bile ducts and extensive fibrosis of liver accompanied by degenerative lesions in pancreas and kidney; and typical bile duct hyperplasia [66]. Previous researchers [97] have also reported that bile duct carcinoma in Khaki Campbell ducks resulted due to impacts of aflatoxicosis. As per the studies in ducks fed (diets spiked with AF) with AFB1, both feed intake and weight gain were reduced but without affecting feed efficiency [99].

While the threshold of clinical toxicity and subclinical toxicity in ducks would normally remain a debatable subject among scientists, the cutoff levels of AFB1 in duck feeds, prescribed in South-east Asia region and that of the West (America & Europe), are likely to vary because of the biotic and abiotic ambiences prevalent in respective regions. While other researchers [100] have cited that even feeding of 300 ppb AFB1 in Pekin duckling diets, for a period of 4 weeks, the loss in weight gain was just insignificant, the Indian studies, including that of author's own lab, have suggested that as much as 10 ppb of naturally arisen AFB1 (or higher) in duckling diets could precipitate in huge morbidity and mortalities in Pekin duck stocks. However, other authors have emphasized that mortalities to the tune of 50% of most ducklings could be witnessed in both Pekin and Khaki Campbell ducks when the naturally arisen AFB1 levels hovered around 20–41 ppb during post-monsoon periods with feeds compounded with grains stored just for 6–8 months [94]. This would mean that naturally arisen AFB1 levels were indicators of rampant and conducive growth of *Aspergillus* fungi, which not only produced AFB1 in locally stored feed, but also might have supported growth of other fungi, leading to co-production of other mycotoxins possibly, with possible increase of mycotoxin cocktails.

Earlier workers reported that duck diets spiked with AFB1 up to 48 ppb actually gave rise to huge brooder-house morbidity resulting in ~20% mortality, poorer FCRs, coupled with geno-toxicities building up within the bone marrow cells of White Pekin ducks [101].

3.3 Aflatoxicosis in turkeys

As has been reported near unanimously, for inter-species susceptibility ranking in decreasing order (Ducks → Turky → Japanese Quails → Chickens) by numerous authors [88, 91, 102–105], the susceptibility profiles of Turkey fall only next to the ducks. The turkey's sensitivity to AFB1 can safely be attributed to its efficient production of AFBO within the system, which is mostly linked to the P450 enzyme that is responsible for AFB1's bioactivation and metabolism within turkey livers. The earlier work in turkey has established well that two turkey-P450 enzymes, encoded by *CYP1A5* and *CYP3A37,* are predominantly responsible for converting AFB1 into AFBO *in vitro* and *in vivo* [105, 106–108]. The complex, i.e., P450 1A5 has high affinity (high Vmax, Kcat; low Km) and catalyzes the production of both *exo*-AFBO and the detoxified metabolite AFM1 according to traditional Michaelis-Menten kinetics. The P450 3A37 is the lower affinity catalyst, exhibiting apparent subunit allostery conforming to Hill enzyme kinetics and producing *exo*-AFBO and AFQ1.

The higher sensitivity of domestic turkey to AFB1 can therefore be attributed to an unfortunate combination of efficient P450 enzymes and dysfunctional GST enzyme system of the host that allows accumulation of AFB1 adducts in the liver. In contrast, as per reports of earlier researchers [109], the effects of AFB1 exposure in North American wild turkeys were almost similar, but less severe than those encountered in domestic poultry. This differential pattern of response may obviously be reasoned out to cumulative genetic changes that might have happened during domestic selection in commercial ones, or even be, just for the wild ones belonging to totally alien genetic background compared with domestic turkey.

Now, coming to impacts of AFB1 on major production parameters of turkeys, it surely impacts the productivity negatively, causing huge economic losses for poultry industry. Dietary exposure to AFB1 led to lower weight gain and absolute body weights in both chickens and turkeys [110, 111]. Reduced feed intake and decreased efficiency of nutrient usage together, thereafter, usually contribute to impaired growth during AFB1 infections. AFB1 lowered the FCR (feed conversion ratio) causing poultry to consume more feed to produce muscle (broilers and turkeys) [8, 99, 111, 112] and eggs (layers) [113].

The initial clinical signs reported during the outbreak of "*Turkey X disease*" included anorexia and weight loss followed by depression, ataxia, and recumbency. Most affected birds used to die within a week or two. But, at the time of death, most morbid birds frequently exhibited: opisthotonos characterized by arched neck, head down back, and legs extended backward [114], and especially these symptoms when exhibited in ducks should be differentially diagnosed from that of duck viral hepatitis, another duck disease where opisthotonos remains a characteristic symptom.

At necropsy, the body condition remained generally good, but there is generalized congestion and edema in the hosts. The liver and kidney were congested, enlarged and firm, the gall bladder was full, and the duodenum remained distended with typical catarrhal content [98, 115, 116]. Along with decreased feed conversion and weight gain, reduced spontaneous activity, unsteady gait, recumbency, anemia, and death [111, 115–117].

Many researchers [118] have summarized the minimal AFB1 concentrations (threshold of AFB1) capable of exerting major effects in different poultry species, which is extracted and placed below for reference. The authors [118] reviewed the lethal thresholds of the AFB limits in feed, in different species, for which limits of hepatic impairment and loss of productivity of these species were reviewed and compiled. In this specific comparative table involving ducks, turkeys, geese, bobwhite quails, peasants beside chickens were enumerated, where the turkeys were mentioned to be the vulnerable most with 100% lethality attained in this species with just 800 ppb of AFB1. Next were the ducks with 1000 ppb, with peasants and geese all attaining lethality at ~4000 ppb, where the chickens again proved to still far from cent percent lethality at the same (4000 ppb). The turkeys again were shown up to attain hepatic impairment just at 400 ppb AFB1, followed by ducks, geese, pheasants with 500 ppb, and the least impairment shown in the chickens at a dose of 800 ppb. The authors put up a summary of 400 ppb or higher in turkeys, followed by 500 ppb in ducks, followed by chickens, and even pheasants, which showed 800 ppb and beyond at the AFB1 doses, which could pull down production. These reviews by these authors obviously brought to fore the inherent species-specific variation in AFB1 handling capacities across such widely diverse species, when the production tended to get compromised along with the hepatic impairments.

3.4 Aflatoxicosis in quails

It has been reported long back that AFB1 in quails decreased feed conversion, egg production, egg weight, hatchability besides negatively impacting exterior and interior egg quality of quail eggs to some extent [119, 120]. Studies conducted by many researchers have recorded that histopathological analysis of aflatoxin-ingested hens revealed AFB1-characteristic lesions in tissues of the liver, kidney, and intestine [121]. Aflatoxicosis was also reported in hens, and the hematological analysis showed the decreased hemoglobin content than that of the control group [122]. However, the Indian experiences from commercial propagation of Japanese quails, thus far (over last two decades), have not been that livid with respect of AF-induced drops in growth and egg production, with largely uneventful reaction from quail growers, with respect to impact of naturally arisen AF in feed, while following recommended toxin binders in quail feeds.

3.5 Aflatoxicosis and productivity losses in chickens

As regards productive performance losses, exposure to aflatoxins lowered the reproductive performance in poultry. In layers fed with AF, age of sexual maturity got increased with expected drop in egg production [113, 123]. Egg quality parameters, including total weight, shape, albumin or yolk percentage, and shell thickness in chickens and quail can be adversely affected by AFB1, although the effects were variable among studies [110, 113, 124–126]. The declines in poultry production traits are often indirect effects of AF reducing the metabolic potential of the liver. It is obvious from the fact that impaired hepatic protein production likely contributes to AF-induced changes within eggs, as the liver is the chief site of synthesis of proteins and lipids, which are incorporated into the egg yolk.

Another extensive review of the AFB1's effect on various physiological systems of the avian species has been compiled by a different group of research workers [127] in one of their monographs for postgraduate students, over recent years. These authors have detailed and cataloged almost all of the organs and systems, where AFB1-induced injurious effects have been reported. Starting from hepatoxic effects, carcinogenic effects, teratogenic effects were individually cited by the authors, in the form of a forward from <www.Poultrysite.com>. Detailed mentions of haematopoetic, neurotoxic, and immunosuppressive effects in the birds have been documented by the authors, where authors have brought together the negative effects of AFB1 in individual physiological systems, happening across the bursa, Spleen, liver, and kidneys, besides impact on nervous system in chickens, which have been vividly documented. These authors have cited the facts of non-homogeneity in body weight of birds besides negative impacts on carcass, dressed weights, and internal organs in the chickens, as the outcome of AFB1-induced negative changes in chickens [127].

4. Detection of aflatoxin in feed

4.1 Aflatoxin extraction from feed samples

The detection and quantification of AFs in feed samples need a well-organized extraction process. AFs are generally soluble in polar protic solvents, for instance, methanol, acetone, chloroform, and acetonitrile. Therefore, the extraction of aflatoxins

involves the use of these solvents such as methane, acetone, or acetonitrile mixed in different ratio with small amount of water [128, 129]. AF determination based on immunoassay technique requires extraction using mixture of methanol-water (8:2) [130, 131].

The extraction of AF is followed by a cleanup step by using immunoaffinity column (IAC) chromatography [132]. The IAC employs the high specificity and reversibility of binding between an antibody and antigen to separate and purify target analytes from matrices [133]. During sample cleanup, the crude sample extract is applied to IAC containing specific antibodies to aflatoxin immobilized on a solid support such as agarose or silica. As the crude sample moves down the column, the AF binds to the antibody and so gets retained into the column. Second washing is normally required to remove the impurities and unbound proteins. This target is achieved by using appropriate buffer with proper ionic strengths. Thereafter, the AF is recovered by using solvents such as acetonitrile, which break the bond between the antibody and the aflatoxin, which are collected as the clean elutes and then quantified, separately.

4.2 Aflatoxin detection methods

The AFs have been detected in food and feed samples according to the method of Official Analytical chemists (AOAC) [134]. The most commonly used methods are based on emission and absorption characteristics such as liquid chromatography mass spectroscopy (LC-MS) [135, 136], thin layer chromatography [137], high-performance liquid chromatography (HPLC) [138], gas chromatography (GC) [139], and enzyme-linked immunosorbent assay (ELISA) [140]. However, the drawbacks of these commonly used methods are that these methods are tiresome, time-consuming and require skilled technical persons for operation. TLC has excellent sensitivities, but it requires skilled technician, pretreatment of sample, and expensive equipment [141, 142]. Further, TLC lacks precision due to accumulated errors during sample application, plate development, and interpretation. Attempts to improve TLC have emerged in to development of automated form of TLC, which is designated as high-performance thin layer chromatography (HPTLC). HPTLC method of determination of aflatoxin has overcome the errors associated with conventional TLC through automation in sample application, development, and plate interpretation. It is worthwhile to mention that currently HPTLC is one of the most efficient and precise methods in aflatoxin analysis [143, 144]. Keeping in view, the requirement of skilled operators, costs of the equipment associated with its bulkiness, and extensive sample pretreatment, the use of HPTLC has been limited to use in laboratory, and its use in field condition is impracticable. Therefore, rapid and robust methods such as polymerase chain reaction (PCR) and nondestructive methods based on fluorescence/near infrared spectroscopy (FS/NIRS) and hyper spectral imaging (HSI) have been evolved as speedy and easy detection of AFs [145]. PCR technique has also been utilized for the molecular detection of AF producing Aspergillus flavus from peanuts [146]. Likewise, the avfa, omtA, and ver-1 genes encoding the major enzymes in AF biosynthesis were utilized as target genes to analyze AFs using multiplex PCR [147]. AFs from *Aspergillus oryzae* isolated from different Korean foods were detected by using PCR, ELISA, and HPLC [148]. Hydrospectral imaging (HIS) uses the integration of both imaging and spectroscopy to record spatial and spectral characteristics of a given sample [149–152]. Visible/near-infrared (VNIR) or short-wave NIR (SWNIR) HSI techniques are feasible for the detection of AFs as well as identification of different fungal species produced in maize [153–156]. The most appropriate analytical method differs according to the nature of detected mycotoxin, e.g., for AFs, ZEN,

OTA, HPLC fluorescence, and LC-MS/MS are commonly used, while for trichothecenes, GC-MS is mainly preferred [157–162].

Aflatoxin toxicity has a potential threat to production of safe poultry products, i.e., egg and meat. This is a permanent concern for the poultry industry, which has led to development of many methodologies for its detection in feed and other products. Toxicity of aflatoxin may occur in very low concentrations; hence, very responsive and trustworthy methods of its detection are the present need for the poultry producers and other scientific organizations dealing with the poultry research. Proper sampling, homogenization, extraction, and concentration of samples are the most common steps in many analytical procedures. Detection methods can be largely classified into qualitative and quantitative ones [158, 163]. Thin layer chromatography (TLC) can be a used for preliminary test for AFs and Ochratoxins [14, 49]. Recently, for a rapid and specific screening determination of mycotoxin type, immunological methods such as enzyme-linked immunoassay (ELISA) and radioimmunoassay (RIA) are the best approaches because they depend on specific antibodies besides their relatively low cost, easy application, and their results could be comparable with those obtained by other conventional methods such as TLC and high-performance liquid chromatography (HPLC) [164–166].

A tabular presentation has been made to summarize the various aflatoxin detoxification methods reported by various research groups, with mention of their relative advantages and disadvantages (**Table 2**).

Class of detection method	Methods	Advantages	Disadvantages	References
Chromatographic based methods	HPLC	Provide accuracy, reliability and high sensitivity	Extensive sample treatment, exhaustive pre- and post-column derivation process to improve sensitivity	[167]
	TLC	Able to detect multiple metabolites in a single test and provide good level of sensitivity	Susceptible to error, need skilled operator, substantial sample treatment and costly equipment	[167]
	HPTLC GC	Sensitive, limited errors, suitable for multi-toxin detection	Non-linearity of calibration, errant responses effects from previous samples and high variability in precision	[167]
	LC	Highly sensitive and adaptable	Slow detection compared to other methods	[167]
	LC-MS/MS	Offers sensitivity, reliability and does not need the immune-affinity clean-up columns	Expensive, tiresome sample preparation, and requires highly trained and experienced operator	[168]
	UHPLC-MS/MS	Good enough for multi-contaminated sample detection, sensible, reliable with minimum use of solvent and rapid analysis	Need trained technician, expensive high matrix effect	[169]

Class of detection method	Methods	Advantages	Disadvantages	References
Immunochemical Methods	ELISA	Provides simple procedure, cheap, rapid and multi sample testing can be done simultaneously	Cross-reactivity, time consuming clean-up process	[170]
	Radio immunoassay	It offers high sensitivity, minimal matrix effect	Involves safety concerns as radioactive elements are used in assay, false-positive possibility, problems in disposal of radioactive waste materials	[171]
Spectrometric-based methods	Fourier-transform near infrared (FT-NIR) Spectrometry	Fast, environment friendly and require less skilled operator	Time-consuming calibration needed	[172]
	Laser-induced fluorescence (LIF) screening method	Suitable for samples with low levels of contamination	Limits its uses as expensive laser materials are used	[173]
	Back-light Test	Suitable for screening purposes	Possibility of false positive cases high, greater dependency on sample size and freshness of samples	[174]
	Ion mobility spectrometry (IMS)	Offer fast detection, simplicity and sensitivity	Results interpretation difficult	[175]

Table 2.
Aflatoxin detoxification methods, their advantages and disadvantages.

5. Novel strategies to reduce toxicity

Due to the soaring preponderance of AFB1 in poultry feed, several approaches are being evolved to counter or eliminate poisoning/toxicity so as to improve safety and palatability of food products. The control strategies/approaches are classified in to pre- and post-harvest techniques. Pre-harvest techniques are inclusion of genetically modified feed materials in poultry feed formulations that are resistant to *Aspergillus* infestations, climatic aggravations, management of pesticide usage, crop rotation, and timing of plantations. The post-harvest strategies include physical methods such as appropriate drying and storage of raw materials, packaging, and usage of preservatives and pesticides. These approaches act as counteractive actions to reduce the quantity of contamination that is introduced to the raw materials, which are to be included in the compounded feed of poultry. However, these approaches are not sufficient in total elimination of AF contamination. So, more post-harvest know-hows are being utilized to detoxify the contaminated feed. These are use of physical processes, chemical/biological additives to reduce or transform AFB1. All these are discussed in detail below under different headings.

5.1 Physical methods

Hand sorting by visible fungi infection is usually found to be an efficient method to reduce AFB1 in maize kernels. On the other hand, this approach is only applicable on an industrial scale using optical sorting equipment [176]. Besides, sieving can be a useful method of reducing AF poisoning as small components such as broken kernels damaged by fungi can be a source of further spoilage [177]. There is computable differentiation in the major and minor diameters, sphericities, densities of maize kernels contaminated with Aspergillus fungi, and healthy kernels without any infestation. Dehulling is also an efficient physical method of removal of AF contamination [177]. Dehulling can remove more than 90% of AF content from maize kernel [178]. The efficiency of removal of external layer of kernels can be much more visible by floating and washing techniques [176, 179, 180]. Reduction of more than ninefold of AF has been achieved by polishing of the rice kernels [181].

The raw materials should be properly dried to contain safe moisture level, i.e., cereal grains such as maize, jowar (sorghum), bajra, and wheat should not contain more than 11–12% moisture; oilseed cakes or meals such as soybean meal, ground nut cake, sunflower cake, cottonseed cake should contain 10–11% moisture; Milling by-products such as rice bran, wheat bran, etc., should not contain more than 11–12% moisture; animal protein sources such as fish meal, meat meal, etc., should not contain more than 9–10% moisture [130]. The storage godown's relative humidity also could influence the moisture content of the feed ingredients, and therefore, proper relative humidity, i.e., <60% should be maintained in the feed storage godown. The ideal temperature during storage should be <15°C. There should be proper cross ventilation in the feed godown, and feed bags should be stored in stacks, over wooden planks or stone slabs allowing a minimum air space of 10 cm from the floor and at least 2–3 feet from the wall to allow removal of moisture from the storage area [182]. The duration of storage also affects the aflatoxin content of the feed (**Table 3**) [183].

A summary on effect of storage duration triggering growth of aflatoxin (in ppb) can be checked here.

Sunlight causes photodegradation of AF leading to significant reduction in AFB1 contents in the feed. More than 60% of AF was documented to be degraded after 30 h exposure of poultry feed to sunlight [184].

In modern feed manufacturing technology, heating treatment is mostly used to degrade mycotoxin to certain extent during processing. AFs are stable at high temperature, and therefore, high heating is required to remove them quantitatively. Many research workers have demonstrated that high temperature (150–200°C) can remove significant amount of AFB_1 (an average of 79%), which is most effective at high humidity [185–188]. Microwave heating is less effective in reducing the AF contamination. The percent of

Storage duration of feed (days)	Aflatoxin content ppb	Percent positive (%)
1–5	7.9	20.5
6.10	8.0	23.4
11–15	10.7	30.0
16–20	27.9	66.7

Table 3.
Effect of storage duration on the aflatoxin content of the mixed feed.

reduction was between 22% and 32% [184]. AF content was significantly reduced in the traditional Mexico food tartilaas by microwave thermal-alkaline treatment [189].

Gamma (ϒ)-irradiation has been demonstrated for food substrates such as groundnuts, grains, soybean, and animal feed. Irradiation by ϒ-ray with high dose (60 KGy) is moderately effective with average reduction of 65% of AF [190–194]. ϒ-irradiation (at a dose level of 5–25 KGy) of chick feed reduced the AFB1 concentration by 32–42% [184].

5.2 Chemical methods

5.2.1 Acidification

Treatment of poultry feed (AF-contaminated) with citric, lactic, tartaric, and hydrochloric acid is found to be very effective in reducing the toxicity particularly when the poultry feed is soaked in acidic solution for a particular period. AF degradation can be observed in 24 h or less when the soaking is carried out at room temperature [188, 195, 196]. On the other hand, some acids such as succinic, acetic, ascorbic, and formic have marginal effect in decreasing the AFB1 toxicity. The detoxification product of AFB1 in acidic medium is AFB2a, which is very less toxic than AFB1. Treatment with citric acid reduced the AFB1 content in duckling feed remarkably, i.e., 86–92%, whereas moderate decrease of about 67% was observed with lactic acid solutions [197, 198].

5.2.2 Ammoniation

Ammoniation (or ammonization) has been used to breakdown AFB1 in an alkaline environment. This technique involves treating contaminated food with gaseous or liquid ammonia (1.5–2%) at room temperature for a time period ranging from 24 h to 15 days approximately. By following this approach, as high as 99% degradation of AF can be achieved [199–201]. Disadvantage of this technique is the requirement of complex infrastructure to conduct ammoniation process, which led to the discontinuation of this technique worldwide [202].

5.2.3 Ozonation

Ozonation is one more novel chemical method to control AF contamination during storage of grains [203]. However, other researchers reported a reduction of 86.75% AFB1 levels in wheat, when ozonolysis was used at a concentration of 6–90 mg/l for 20 min [120]. A variety of food substrates have been investigated with ozone, indicating its effectiveness in reducing the AFB1 in many feedstuffs [153–158, 204–210]. Ozone can destroy AFs efficiently (up to 66–95%) of the initial concentration in cereal grains and flours, soybean, and peanut [211–213].

5.3 Biological method

Reduction in AFB1 is observed probably due to metabolism or by physically binding of AFB1 directly when food substrates are inoculated with strains of a particular bacteria, fungi, or yeast. Two *Lactobacillus amylovorus* strains and one *Lactobacillus rhamnosus* strain removed more than 50% AFB1 rapidly after 72 h of incubation. L. *rhamnosus* strain (LC-705) can significantly and very quickly remove approximately 80% of AFB1 from culture media, which is dependent on temperature as well as concentration of the bacteria [214]. The GG strain of *L.rhamnosus* reduced the AFB1

contamination by 54% in the soluble fraction of the luminal fluid within a time of 1 min compared with *L. rhamnosus* LC-705, which removed 44% AFB1 under similar conditions [215]. There was 72% reduction in uptake of AFB1 by the intestinal tissue in presence of *L. rhamnosus* strain GG compared with 63% and 37% by *Propionibacterium freudenreichi spp*. *Shermanii JS* and *L. rhamnosus* strain LC-705, respectively. AFB1 degradation as high as 80% has been reported by using several genera of bacteria, yeast, and fungi such as *Lactobacillus*, *Saccharomyces*, *Cellulomicrobium,* and *Pleurotuseryngii* with treatment time up to several days [216–221]. Addition of *Saccharomyces cerevisiae* CECT strain to drinking water of broilers fed AFB1-contaminated diet (1.2 mg/kg) resulted in significant improvement related to production and biochemical parameters, hepatotoxicity, and histopathology of liver [222]. Fungal strains such as Aspergillus niger, Eurotiumherbariorum, a Rhizopus spp., and non-aflatoxin producing *A.flavus* were able to convert AFB1 to aflatoxicol-A (AFL-A); and then AFA-L was converted to aflatoxicol-B (AFL-B) by the actions of organic acids produced from the fungi. These AFA-A and AFA-L compounds are nontoxic indicating the significant role of fungi in detoxifying AFB1. Rhizopus oligosporus was able to inhibit or to degrade AFB1 when cultured together with AFB1-producing fungi *A.flavus* [223]. Botanical extracts such as aqueous extracts of various plants species to dissolve AFB1 have been studied to determine percent degradation after incubating the toxin in this aqueous extract for a time period of 24–72 h. The extracts from Adhatodavasica Ness and Corymbiacitridora achieved >95% degradation of AFB1 [224–227]. However, active components in these plant extracts responsible for this degradation need to be identified, which could prove useful for increasing the efficiency of this method of reduction of AFB1 in poultry feed. The potential of purified enzymes from various biological sources has been investigated for AFB1 degradation. These enzymes are laccases, manganese peroxidase, and Bacillus aflatoxin-degrading enzyme. The efficacy of this strategy is very high, but they have not been tested on food substrates, so the efficacy on food products is still unknown [202]. The time of enzyme treatment is high, which may take several days to complete the process. Therefore, this method may not be practicable for large-scale applications [228–231].

5.4 Nutritional supplements method

A number of feed supplements have provided protection against the damage caused by AFB1. Fat-soluble vitamins such as vitamin A, E, K, and D could be used in preventing the toxic effect of aflatoxins [232]. Supplementation of vitamins A, E, and C has resulted in enhanced antioxidative effect in poultry birds and protects the immune cells from oxidative damage induced by AFB1 [233]. Many studies conducted worldwide have been compiled together in a broad meta-analysis in poultry, where the nutritional supplements are exploited against AFB1 in broilers, and some of them could be well deliberated as well organized and useful to improve the adverse effects ofAFB1 [234]. Selenium (Se) and zinc (Zn) are two understudied trace elements for their protective roles against oxidative stresses and other adverse effects induced by AFB1. A number of studies have documented the importance of Se and Zn in human and animal biology when used optimally. Selenium is an essential nutrient of fundamental importance inhuman and animal biology. Se is a significant feed-derived natural antioxidant in poultry, and adequate level of Se is crucial for chicken health, productive and reproductive characteristics (embryonic development and sperm quality), and optimal functioning of immune system [235]. Two major Se sources, which are inorganic (selenite orselenate) and organic selenium (seleno-methionine),

are used in poultry [236]. AFB1 exposure induced liver dysfunction by disturbing the tissue enzyme activity and enhanced apoptosis, but the Se administration protected liver tissues against AFB1-induced toxicity [237]. A number of studies conducted on various organs in poultry birds demonstrated the protective effects of Se against AFB1 [238–240]. The dietary sodium selenite in the feed of broiler has excellent effects on oxidative stress and apoptosis and can amend the immunosuppression effects induced by AFB1 in spleen of broiler [239]. Se supplementation has improved AFB1-induced apoptosis at a concentration of 0.4 mg/kg [240]. Further, Se supplementation in broiler diet provided protection against AFB1-induced changes in the ileum, and sodium selenite improved the cellular immune functioning of the AFB1-affected ileum mucosa [238]. Se inhibits AFB1-DNA binding and adducts formation and sodium selenite and Se-enriched yeast extract protect cells from AFB1 cytotoxicity [241]. Out of all the functions, antioxidant and anti-tumor abilities are the most important roles played by Se. Se may prevent the binding of DNA with carcinogens as well as reactive Se metabolites can render the carcinogens into non-carcinogenic compounds. Dietary Se has been shown to protect chicks from AFB1-induced liver injury by inhibiting CYP450-enzyme, which is responsible for the activation of AFB1 to toxic AFBO [242]. Zinc (Zn) is known for its beneficial effects on humans and animals for many decades due to its principal role in individual's growth, development, and optimal functioning of various physiological processes. Certainly, the past two decades have seen a fast growth in knowledge of the fundamental mechanisms, whereby Zn put forth its universal effects on immune function, disease resistance, and general health [243–245]. Although a number of studies have been carried out on AF-induced systemic toxicity in poultry, signifying protective effects of zinc against a range of noxious agents in human and different laboratory animal [246–249]. Only few studies focused on defensive effects of Zn against AFB1. Zn supplementation in AFB1-intoxicated birds significantly enhanced the growth performance of poultry birds in terms of higher body weight gain and better feed efficiency [250]. The function of Zn in enhancing various systems of the body could be used as modifying means against AFB1 intoxication.

5.5 Addition of adsorbents method

The best way to neutralize aflatoxins already present in feed is by binding them to an inert compound before they are absorbed from the intestine. One of the methods of detoxification of aflatoxin is the use of non-nutritive adsorptive materials in the diet to reduce aflatoxin absorption from the gastrointestinal tract. When an adsorbent is added to the feed, it adsorbs the aflatoxins in the gastrointestinal tract (GIT) and safely excretes in the feces; and thereby it prevents absorption and transport to the target organs. Hence, the final effect of addition of adsorbent is reduction in the dose of absorbable toxin to a concentration that does not affect the performance adversely. The use of activated charcoal as an oral remedy for the management of toxicity is well recognized. Charcoal acts as an insoluble carrier that non-specifically adsorbs molecules, thereby preventing their absorption [251]. The efficacy of activated charcoal in binding AF has been demonstrated by many research workers [252–255]. Addition of 200 ppm of activated charcoal to broiler diet contaminated with 0.5 ppm aflatoxin provided protection to broilers against harmful effects of AF on performance and biochemical parameters [256]. Dietary addition of super-activated charcoal @ 0.5% was marginally effective in ameliorating some of the toxic effects associated with AF, i.e., diet contaminated with 4 mg AF [251]. Addition of esterified glucomannan

(EGM) in broiler diet significantly decreased the harmful effect of AF contamination (300 ppb) [257]. Dietary supplementation of esterified glucomannan (0.05%) was effective in ameliorating the toxicity of naturally contaminated diet containing Aflatoxin 168 ppb, ochratoxin 8.4 ppb, zearalenone 54 ppb, and T-2 toxin 32 ppb [258]. Dietary addition of super-activated charcoal (SAC) @ 0.5% of diet was marginally effective in counteracting the toxic effects associated with chronic toxicosis in growing broilers. The protective effect probably involves the sequestration of the toxic molecules in the gastrointestinal tract and chemisorptions to the charcoal, which suggests that SAC is highly variable in its ability to ameliorate the toxic effects of AF in growing broilers and to bind AF in *vivo* [251]. The weight of broilers increased by 63–100% by addition of activated charcoal, bentonite, and fuller's earth to aflatoxin-contaminated feed (120 μg/kg feed). However, bentonite addition was more effective in counteracting histopathological effects compared with activated charcoal and fuller's earth [259]. A commercial binder, which is an extra-purified clay containing diatomaceous earth mineral, antioxidants curcuminoids extracted from turmeric and enzymes (Epoxidase and esterase), was added @ 0.2% to broiler chicken diet contaminated with 0.6 ppm AFB1. The addition of binder could significantly counter the harmful effects (depressed body weight, increased feed intake, and poor FCR). On the other hand, the beneficial effect on nutrient digestibility and gut function of broilers does not get confirmed [260]. Supplementation of diatomaceous earth, sodium bentonite, and zeolite at level of 0.5% or 1% individually or in combination to a 300 ppm aflatoxin B1-contaminated broiler feed was effective in improving the harmful effects of aflatoxin toxicity on the liver and livability percentage in broiler chicks. Nonetheless, sodium bentonite and zeolite were more efficient than diatomaceous earth in ameliorating the toxicity [261]. Efficacy of sodium calcium alluminosilicate, curcumin derived from turmeric (Curcuma longa), and sodium bentonite has been proven beneficial in ameliorating the adverse effects of AF on broiler chicks and growing poultry [262–265].

Further, a summary of various studies employing different detoxification methods to control aflatoxin is placed in the cited table for ease of interpretation, with relevant references (**Table 4**).

5.6 Emerging novel approaches to overcome aflatoxicosis: Genetics vs epigenetics

Genetic approaches to control aflatoxicosis can be straightforward, which can rely on a genetic selection to bring in tolerance in the host (poultry) to moderate or high levels of dietary aflatoxins. The experiences available at the ICAR's duck research and breeding facility at Bhubaneswar, India, provide some promising trends in this direction. The primary breed and strain differences evident in ducks (CARI, RC's studies) do sustain a promise that through long-term selection program or by prudent cross-breedings, the commercial ducks could be rendered tolerant to moderate dietary aflatoxins (40–50 ppb levels), as the native ducks are seen to tolerate such sporadic toxin spurts, better than Khaki Campbells and White Pekins [64], without exhibiting much morbidities in layers. However, both ethics and practicality could discourage such a selective breeding approach against aflatoxicosis, unless safeguards are in place to prevent significant residues generated within the birds from spiking of their diets with AFs, from being passed on to any of the public food chain, through landing of such genetic stocks in the consumer market inadvertently.

Marks and Wyatt (1980) were the first such team of workers [270] who have observed different mortality patterns resulting from acute aflatoxicosis in various

Detoxification method	Specific agent used	Amount of agent	% Reduction in AFB1	Initial Amount of AFB1 (ng/g)	Substrate	Treatment Time (min)	References
1. Physical method	Heat	200°C	97	100.2	Wheat	30	[186]
a. Temperature	Heat	150°C	81.2	10	Soybeans	90	[188]
	Heat	150°C	78.4	237	Peanuts	120	[185]
b. Irradiation	ϒ-irradiation	25 kGy	42.7	192.1–894	Chick feed	-	[135]
	ϒ-irradiation	15 kGy	18.2	25	Poultry feed	-	[190, 191]
	ϒ-irradiation	8 kGy	60.26	50.38	mix. maize	-	[194]
c. Organism	Lactobacillus	$2.5X10^{10}$	71	10 ng/ml	Aqueous	-	[219]
	rhamnosus	CFU/ml	29.9–44.5	50–500 ng/g	solution	-	[221]
	L. plantarum	10^8 CFU/ml	93.12	50 ng/ml	Maize	72 h	[218]
	L.acidophilus	$7X10^9$	86	128 ng/g	Cereal	28 days	[216]
	Pleurotus eryngii	CFU/ml 3 g			maize		
2. Chemical method	Citric acid	1 N	97.22	4–30 ng/g	Rice	15 min	[196]
a. Acidification	Citric acid	1 N	94.1	7.6 ng/g	Soybean	18 h	[188]
	Acetic acid	1 M	12.5	200 ng/ml	Aqueous solution	24 h	[195]
b. Ammoniation	Ammonia	1.50%	99.3	750 ng/g	Maize	13 days	[199]
	Ammonia	2.00%	52.7–67.7	17–7500 ng/g	Maize	60 min	[201]
	Ammonia	2.00%	88.02	4000 ng/g	Maize	24 h	[200]
c. Ozonation	Ozone	40 ppm	86.75	10 ng/g	Wheat	20 min	[204]
	Ozone	21 mg/l	25	180 ng/g	Peanuts	96 h	[206]
	Ozone	50 mg/l	89.4	189.53 ng/g	Peanuts	60 h	[207]
	Ozone	75 mg/l	78.8	53.6 ng/g	Cornflour	60 min	[209, 210]
	Ozone	90 mg/l	88.1	83 ng/g	Maize	40 min	[209, 210]
	Ozone	60 mg/l	65.9	200 ng/g	peanuts	30 min	[205]
3. Combination of methods	ammonia + heat	2% ammonia + 121°C	99	17–7500 ng/g	Maize	120 min	[201]
a. Ammoniation + heat	ammonia + heat	2% ammonia + 121°C	99.9	4000 ng/g	Maize	15 min	[200]
c. Alkalization + heat	NaOH + heat	NaOH to PH 10 + 98°C	97	34.50 ng/g	Dried fig	60 min	[266]

Detoxification method	Specific agent used	Amount of agent	% Reduction in AFB1	Initial Amount of AFB1 (ng/g)	Substrate	Treatment Time (min)	References
d. Acidification + heat	HCl + heat	5 M HCl + 110°C	100	45.68 ng/g	Maize	4 h	[267]
	Lactic acid + heat	1 M Lactic acid + 80°C	85.1	1000 ng/g	Aqueous	120 m	[268]
	Citric acid + heat	0.1 g/ml citric acid + 120°C	93.1	383 ng/g	solution pistachios	60 min	[269]

Table 4.
Summary of studies using various detoxification methods to degrade AFB1.

growth-selected lines of Japanese quails of USDA experimental facilities in 1980s. This observation had thereafter led to the genetic selection of Japanese quails for resistance to acute aflatoxicosis by breeding survivors from a population of quails, which were given a single oral dose of aflatoxin that resulted in high mortality [271]. After five generations of selection, an 11-fold increase in resistance was attained in one of the aflatoxin-resistant lines. The next group of workers observed genetic variation in certain physiological parameters of selected commercial broiler populations and suggested the feasibility of genetic selection of chickens for infusing resistance to aflatoxicosis [272]. Many other researchers had observed genetic variation in a non-selected population of chickens [273].

The other successful directional selective breeding for AFB1 resistance was also reported [274]. Under their breeding trials, two populations of broiler chickens [Athens-Canadian (AC) versus another broiler commercial stock] were subjected to genetic selection for resistance to aflatoxicosis by exposing the respective chickens from each of the stocks (two) with a single oral dose of aflatoxin, which was capable of resulting in 40–70% mortality, otherwise. A simultaneous, non-selected control group was also maintained, which was not exposed to any AFB1. As for the selection method, the birds surviving the aflatoxin challenge were propagated as breeders, for subsequent generations. According to the outcome of their study, rapid progress was visible within the AC population for resistance to aflatoxin, whereas only moderate progress for AFB1 resistance was attained in the commercial broiler stock. After five generations of selection in the AC population, LD50 values of 9.42 and 17.05 milligrams aflatoxin per kg body weight (BW) were determined for both the non-selected and selected lines. Similarly, after four such generations of such selection in above commercial broiler population, LD50 values of 6.05 and 8.02 mg aflatoxin/kg BW were determined for the non-selected and selected lines, respectively. These experiments demonstrated that genetic progress for AFB1 tolerance could be achievable in chickens, but the quantum of such progress for resistance to AFB1 could be influenced by the population's background, meaning response to such genetic selection for such AFB1 resistance or tolerance was always a subject of genetic constitution of the hosts.

On a practical front, there have been couple of studies that attempted direct breeding of ducks for tolerance or resistance to AFB1's presence in diets, on selective-breeding platforms, way back in 1980s, after which very little progress has been registered in duck-producing countries. The obvious interpretation could be that ensuring a diet with minimal cutoff levels for AFB1, which was achievable using toxin binders, mold inhibitors, etc., was probably preferred to (better than) raising stocks with resistance to AFB1.

5.6.1 Epigenetic studies on aflatoxicosis

Epigenetics is the study of heritable phenotypic alterations caused due to change in chromosomal topology rather than change in DNA sequence [275, 276]. The underlying epigenetic processes such as chromatin remodeling, non-coding RNAs (micro RNAs), DNA methylation, acetylation, deacetylation, histone modification, etc., are affected by prolonged exposure to aflatoxin, causing alteration in protein synthesis and thereby the gene expressions. Aflatoxin-B1 mainly induces DNA methylation, which plays a critical role in the development of all most all cancer types owing to its silencing effect on tumor suppressor genes [277]. In this process, the fifth carbon of the cytosine in dinucleotide 5'-CG-3′ is selectively methylated to form 5-mC [278, 279]. Aberrant methylation of promoters in eukaryotic cell may lead to silencing

of regulatory genes especially tumor suppressor genes and thereafter, affect their signal pathways and lead to development of disease and cancers. Alteration in cellular epigenome compromises genomic stability and alters gene expression, which thereby affect the central dogma of molecular biology and ultimately phenotypic characters.

Few human studies have been reported in literature detailing the epigenetic changes which accompany aflatoxin-exposure, across various vital organs such as white blood cells, egg yolk, plasma, etc. It has been reported that maternal exposure to aflatoxin during early embryonic development leads to formation of aflatoxin albumin (AF-alb) adducts and genome-wide differential DNA methylation patterns of white blood cells for 71 CpG sites, including in genes related to growth and immune function [280]. It has been reported to cause various types of cancers such as colon cancer [281], sarcomas [282], lung cancer [283], ovarian cancer [284], leukemia [285], urological cancer [286], breast cancer [287], Hodgkin lymphoma [288], including cardiovascular diseases [289] and schizophrenia [290].

Though epigenetic approaches raise hopes for a long-term strategy to overcome aflatoxicosis problems in ducks, the literature is just hollow, except some rudimentary reports. The ICAR-DPR's own annual report [95] indicated that most significant aflatoxicosis-induced production losses peaked and precipitated only in alternate generations/years, despite emergence of naturally arisen dietary aflatoxins (10–50 ppb ranges throughout the year) since last decade, which suggest that epigenetic sensitization of the ducklings/ducks every generation at early or perinatal stages, which are usually the phases of methylation-induction processes in an epigenetic regime. The RC, CARI (now a regional Station of Directorate on Poultry, Research, Bhubaneswar, India) has just concluded a large-funded program on epigenetics research in ducks, which has shown positive feedbacks through better egg-production recorded from AF-sensitized ducks versus the controls, thus signifying feasibility of such approaches in coming decades.

6. Conclusions

Almost all classes of poultry are physiologically vulnerable and susceptible to aflatoxins, especially the AFB1, which produces acute, chronic, mutagenic, and teratogenic toxicity along with causing millions of dollars per year damage to the poultry industry, worldwide. The high frequency and levels of AFB1 recently found in food supplies, particularly, poultry feed of various countries indicate wide exposure of poultry birds to this toxin, which still remain uncontrolled. The most appropriate analytical method differs according to the nature of detected mycotoxin, e.g., for AFs, ZEN, OTA, HPLC fluorescence, and LC-MS/MS are commonly used, while for trichothecenes, GC-MS is mainly preferred. Due to the increasing abundance of AFB1 in poultry feed, several approaches are being evolved to counter or eliminate poisoning/toxicity so as to improve safety and palatability of food products. Between pre- and post-harvest strategies, there are many options available to reduce the toxicity to a great extent. Large-scale implementation of these techniques could make a large impact worldwide to reduce the aflatoxin related toxicities such as growth impairment, histopathology of organs, and immunosuppression in poultry birds. Development of suitable method for detection of aflatoxin in field level and environment-friendly detoxification keeping in view the food safety will be beneficial strategies for achievement of poultry products, which will be safe and secured for human consumption. Quality control of feed ingredients; prevention of fungal growth with reduction in concomitant aflatoxin production; use of efficient detection method and suitable environment-friendly detoxification

methods, are essential to the feed manufacturers to reduce the exposure to aflatoxin and to make the poultry production a profitable enterprise. Among various classes of AFB1-susceptible poultry species, recent research on epigenetics in ducks has shown some positive feedbacks regarding feasibility of such approaches in upcoming decades, while needs to develop poultry species genetically resistant to Aflatoxins through direct selection may not find a great favor from primary breeders anymore, in twenty-first century despite promising results documented during late-twentieth century.

Author details

Surya Kanta Mishra* and Bijaya Kumar Swain
ICAR-Directorate of Poultry Research Regional Station, Bhubaneswar, Odisha, India

*Address all correspondence to: suryakmishra@yahoo.com

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