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Review Article

Aflatoxins in feeds: Issues and concerns with safe food production

S. Thakur[1], R. K. Singh[1], P. S. De[2] and A. Dey[1*]

[1]Division of Animal Nutrition and Feed Technology, ICAR- Central Institute for Research on Buffaloes, Hisar - 125 001, Haryana, India; [2]Department of Botany, Sonamukhi College, Bankura University, Bankura - 722 207, West Bengal, India

Abstract

Aflatoxin is a category of mycotoxin produced by the fungal species *Aspergillus flavus* mainly, besides *Aspergillus parasiticus*, *Aspergillus pseudocaelatus*, *Aspergillus pseudonomius* and *Aspergillus nomius*. The hot-humid tropical climatic conditions predispose certain crops to mould growth, especially maize, peanuts, cottonseed and dairy products. Aflatoxin contamination of animal feeds causes huge economic loss to the feed-food industry throughout the world. Aflatoxin-B_1 is the most hazardous toxin and can be transferred from the feed to the milk of ruminant animals as aflatoxin-M_1 and ultimately poses a significant human health hazard owing to its hepatotoxic, immunosuppressive and carcinogenic effects. In India, the Bureau of Indian Standards has set a maximum permissible level of 20 ppb (aflatoxin-B_1) in all animal feeds; however, the Food Safety and Standards Authority of India recommends a permissible limit of 0.5 ppb in human foods. Aflatoxins can be transferred (0.8 - 6.5%) from the feed to the milk of animals as aflatoxin-M_1 and ultimately pose a significant human health hazard. Not only in milk, but the presence of aflatoxin in other dairy products, eggs and edible animal products also pushed the formulation of regulations to minimize the exposure of aflatoxin to food animals. Pre- and post-harvest prevention of aflatoxin production in agricultural products is necessary with improvement in management protocols for reducing the susceptibility of mould growth. Proper storage conditions, avoiding mechanical and insect damage, maintaining temperature, aeration and moisture are important for reducing aflatoxin contamination of feeds. Various acids, viz. sorbic acid and propionic acid, are used commercially at adequate levels to reduce aflatoxin contaminations in products. Gamma-irradiation, chemical degradation, microbial inactivation and reduction of toxin bioavailability by selective chemisorption with clays are few techniques to reduce the aflatoxin levels in animal feeds. Provision of good quality protein balanced diet with supplementation of N-acetylcysteine, choline, methionine and vitamin E is advantageous for reducing the severity of aflatoxicosis. Sustainable preventive strategies for pre-and post-harvest management of food-feed crops need to be taken up to reduce aflatoxin exposure to animals for the production of safe foods of animal origin.

Key words: Aflatoxins, Animal feeds, Animal products, Food safety, Hepatotoxicity, Milk

Highlights

- Aflatoxin contamination of animal feeds causes huge economic loss to the feed-food industry.
- Aflatoxin levels in milk and milk products, meat and meat products, and eggs are alarming for human health and the economy of the country.
- Pre- and post-harvest management of feeds are important tools for aflatoxicosis prevention.
- Avoiding conditions suitable for aflatoxin formation and evading toxin consumption is paramount.
- Upper safety limit of aflatoxins in feed should be regulated to a narrow margin.

Introduction

Mycotoxins are globally addressing concerns for food safety and ultimately threaten human health. The term 'mycotoxin' comes from the Latin word "toxicum", which means poison. Agricultural importance and economical concern category of mycotoxins i.e. aflatoxin (Detrimental substances produced by certain moulds) originated from the fungal species *Aspergillus flavus* (FAO, 1991). The word was coined around 1960 after its

*Corresponding Author, E mail: avijitcirb@gmail.com

correlation as an etiological agent with Turkey X disease characterized by liver necrosis (Dhakal and Sbar, 2021). Apart from *Aspergillus flavus*, other mould species such as *Aspergillus parasiticus*, *Aspergillus pseudocaelatus*, *Aspergillus pseudonomius* and *Aspergillus nomius* are also mycotoxins producers (Iqbal *et al*., 2015). Certain crops such as maize, peanuts, cottonseed etc. including fodder crops of maize and sorghum besides dairy products are natural habitats of aflatoxin producing fungi (WHO, 2017). Tropical climatic conditions (hot-humid climate) exacerbate aflatoxicosis. Although, the loss owing to aflatoxin contamination of animal feeds is not calculated separately in India, the climatic conditions of the southern USA cause huge economic loss to the U.S. corn or maize industry and were estimated at around US$ 1.68 billion annually (Mitchell *et al*., 2016).

Aflatoxins B_1, B_2, G_1, G_2, M_1 and M_2 are major classes of aflatoxin (based on their fluorescence under UV light (blue or green) and relative chromatographic mobility during thin-layer chromatography) (Khalid and Imran, 2019) concern to human health hazards. Among various types of aflatoxins, aflatoxin B_1 is the most hazardous toxin, and BIS regulates its upper safe limit in a very narrow margin i.e. 20ppb in most of animal feedstuffs (BIS, 2021). Aflatoxins can be transferred from the feed to the milk of ruminant animals as aflatoxin-M_1 and ultimately poses a significant human health hazard (FSSAI, 2020). Not only in milk, but the presence of aflatoxin in other dairy products, eggs and edible animal products also pushed the formulation of regulations to minimize the exposure of aflatoxin to food animals. Aflatoxin has been detected even in pasteurized ultra-high temperature milk, broiler chicken meat, eggs and sausage (Siddappa *et al*., 2012; Shaltout *et al*., 2014). In ruminants, chronic exposure to dietary aflatoxin-contaminated feeds can suppress milk production and impair reproduction. The hepatotoxic nature of aflatoxin inhibits immune function and increases susceptibility to diseases. Apart from huge economic impact, aflatoxins are potent carcinogens and are addressed as a significant human and livestock health hazard (Abbas *et al*., 2010). As the demand for animal products is increasing with the rising human population and there is a shortage of quality animal feeds, the safe food production from animal origin is of great challenge. The article is intended to overview the aflatoxin contamination of animal feeds and their relationship with safe food production from animal origin.

Feeds susceptible to aflatoxins

Aflatoxins producing fungi, *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nominus* are ubiquitous in hot and humid conditions in subtropical and tropical countries. In tropical countries, warm humid climates can give rise to toxigenic mould growth and aflatoxin production. Aflatoxin can be produced by fungi at the pre-harvest stage as well as post-harvest storage, which makes it a challenging task to combat aflatoxin contamination of feeds. In susceptible plant species, *A. flavus* and *A. parasiticus* are weak pathogens of the reproductive organs and are aggressive in mature seeds with high oil content. About one-fourth of the world's food crops are estimated to be affected by mycotoxins (Kumar *et al*., 2008), and many countries of Asia, Latin America, sub-Saharan Africa reported aflatoxin as the major contaminants of maize, wheat, rice, barley and oilseeds, including peanut, sunflower, soybeans, mustard, cottonseed and their products (Filazi and Sireli, 2013; Lee *et al*., 2015). This might lead to huge economic losses for developing countries, especially due to lack of drying equipment and particularly humid conditions. Various commercially important crops like groundnut, maize, barley, copra, cottonseed, rice, wheat, legumes and their byproducts and spice crops are susceptible to fungal attack. Data collected from various studies about aflatoxin concentration in various feeds are described in Table 1.

Table 1. Various feedstuffs and their reported aflatoxin concentrations

Feedstuffs	Country	Aflatoxin B_1 (µg/kg)	Reference
Maize	Zambia	16	Kachapulula *et al.*, 2017
Maize	India	62	Kotinagu *et al.*, 2015
Maize	China	33	Xiong *et al.*, 2018
Maize silage	France	7.0–51.3	Richard *et al.*, 2009
Maize silage	Iran	10.98	Ehsani *et al.*, 2016
Corn gluten meal	Pakistan	57.8-89.9	Fareed *et al.*, 2014
Groundnut cake	India	23.3	Kotinagu *et al.*, 2015
Groundnut cake	China	47.9	Xiong *et al.*, 2018
Cottonseed cake	India	23.3	Kotinagu *et al.*, 2015
Guar meal	Pakistan	23	Anjum *et al.*, 2012
Soybean cake	India	50	Kotinagu *et al.*, 2015
Sunflower meal	Pakistan	33	Anjum *et al.*, 2012
Sunflower deoiled cake	India	10	Kotinagu *et al.*, 2015
Alfalfa hay	Iran	10.74	Ehsani *et al.*, 2016

Maize grain is considered to be a very good substrate for mould infestation especially during water stress, which causes increased aflatoxin levels before harvesting. ICMR conducted a multicentric study analyzing 2074 maize samples from various rural and urban areas of India and found that about 26% of samples exceeded the limit of 30 ppb (Bhat *et al.*, 1996; Bhat *et al.*, 1997). Maize silage is also vulnerable to aflatoxin contamination, as poor storage conditions during ensiling especially in mature silage and various factors like condensation, insect infestation, heating and rainwater leakage might lead to the growth of toxigenic fungi and mycotoxin production (Richard *et al.*, 2009).

In a semi-arid tropical environment, aflatoxin contamination of groundnut is a massive problem in rainfed agriculture. A study conducted by ICMR that included various rural and urban regions of 11 states in India showed that out of 2062 groundnut samples, 21% exceeded the limit of 30 ppb (Bhat *et al.*, 1996). In a study, Waliyar *et al.* (2003) concluded that more than 75% of groundnut cake samples, a major feed ingredient for livestock, contained more than 100 ppb aflatoxin, leading to a high level of aflatoxin M_1 in milk.

Oil-producing seeds are ideal substrates for the growth of toxigenic fungi and feeds like cottonseed cake have been shown to be susceptible. In a study conducted in Pakistan on 110 cottonseed cake samples, 71 samples had higher aflatoxin levels than the minimum level set for animal feeds, with total contamination frequency and mean levels of 88% and 89 µg/kg, respectively (Shar *et al.*, 2020). On the other hand, soybean is considered to be resistant to aflatoxin contamination, as only 14.4% soybean meal samples were found to be contaminated, as compared to maize and oilseed cakes which had higher incidences of contamination (Dhavan and Choudary, 1995). The same study described that the incidence levels of aflatoxin contamination for maize, deoiled rice bran, groundnut cake, sunflower cake, maize gluten, alfalfa forage and fish meal were 65.31, 33.57, 96.35, 75.00, 85.71, 10.91 and 7.00 per cent, respectively.

Permissible limits of aflatoxins in various feedstuffs

The U.S. Food and Drug Administration (FDA, 2019) has set the permissible limit for aflatoxin M_1 (AFM_1) in milk and other dairy products at 0.50 µg/L, while the maximum level of total aflatoxins (AFB1+AFB2+AFG1+AFG2) is 20 µg/kg in feed ingredients offered to dairy animals and foods intended for human consumption (FDA, 2019). The European Commission is stringent and set up an action level for AFM_1 of 0.05 µg/L in liquid milk, AFB_1 of 20 µg/kg in all feedstuffs, 10 µg/kg in complete feeds, and 5 µg/kg in complete feeds for dairy animals (European Commission, 2006). In India, the Bureau of Indian Standards (BIS) has set a maximum permissible level of aflatoxin B_1 at 20 µg/kg or 20 ppb in all animal feeds. However, the Food Safety and Standards Authority of India (FSSAI, 2020) recommends the maximum permissible limit of aflatoxin B_1 in ready to eat oilseeds and oils, 10 µg/kg and aflatoxin M_1, 0.5 µg/kg in milk for human consumption.

Metabolism of aflatoxins

Modes of absorption and transmission: There are four naturally occurring aflatoxins, B_1, B_2, G_1 and G_2 along with other aflatoxins that arise as metabolic products of microbial, human, or animal metabolic systems. Out of these, aflatoxin B_1 is the most toxic and carcinogenic (Waliyar *et al*., 2015). Aflatoxins mainly enter the mammalian system through ingestion of contaminated feedstuffs and foods, and the primary route of absorption is from the alimentary tract. There are also other ways of intake like through mother's milk, trans-placental transfer and inhalation in the form of mycelial fragments, grain dust or fungal spores. Aflatoxins are absorbed efficiently from the gastrointestinal tract by passive diffusion and are mainly transferred to hepatic portal blood (Veldman *et al*., 1992). Aflatoxin B1 (AFB_1) and aflatoxin M1 (AFM_1) have been detected in venous blood 30 minutes after dosing and reached maximum levels at 48h after dosing, which indicates that aflatoxins are absorbed through the rumen rapidly (Zhang *et al*., 2019).

Metabolism and action: The metabolism of aflatoxins differs among different animal species and even individual animals in some cases (Dohnal *et al*., 2014). Biotransformation of AFB_1 involves five mechanisms which involve reactions of reduction, hydration, epoxidation, hydroxylation and ortho-demethylation. In the soluble fraction of the liver, an NADPH-dependent cytoplasmic enzyme (microsomal mixed function oxidase enzyme belonging to the family CYP-450 enzyme system) causes reduction of AFB_1 and produces aflatoxicol. Although aflatoxicol is less toxic than AFB_1, the conversion is reversible, and aflatoxicol can act as a reservoir of toxicity of AFB_1 in the intracellular space (Heidtmann-Bemvenuti *et al*., 2011). Aflatoxicol may also be converted to aflatoxin M_1 and aflatoxin H_1. Metabolic detoxification or hydroxylation of aflatoxin B_1 leads to the formation of aflatoxin M_1, which is then excreted in the milk of lactating humans and animals that have ingested aflatoxin contaminated foods and feeds (Fink-Gremmels, 2008). Various reactions as mentioned above may lead to formation of both toxic [(such as aflatoxin B1-8,9 epoxide (AFBO)] or relatively non-toxic forms like aflatoxin P_1 (AFP1), aflatoxin Q1 (AFQ_1) or hemiacetal form of aflatoxin B1 (AFB_{2a}) (Wu *et al*., 2009).

Although AFB_1 does not have mutagenic activity in its pure form, it is converted into a potent carcinogen through epoxidation reaction, as a consequence of which it can react rapidly and covalently with nucleophilic sites of macromolecules like deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and proteins (Lanier *et al*., 2020). The compound 8, 9 epoxide of aflatoxin is responsible for the reduction in protein synthesis and the mutagenic, teratogenic and carcinogenic effects. AFM_1 and aflatoxin Q1 (AFQ_1), products of hydroxylation reaction of AFB_1, have hydroxyl group which helps in their conjugation with glucuronic acid, sulfate and glutathione, increasing their water solubility in the process and facilitating their excretion in urine, bile and milk. The extent of conversion of B-toxins to M-toxins differs among species (Sergent *et al*., 2008). As per calculations, a baby of 2.5 kg at one week of age would be exposed to 10 ng AFM_1 per kg body weight on average consumption of milk with a contamination concentration of 50 pg aflatoxin per ml milk (Wild *et al.*, 1987).

Excretion of aflatoxin metabolites: There are various routes of excretion of aflatoxins such as faeces, urine, bile, semen, milk and eggs. AFM_1 and AFP_1 have been detected in dog urine (Bingham *et al.*, 2004). Aflatoxicol, AFB_1 and AFM_1 are excreted in the egg. Humans excrete aflatoxin M1 (AFM_1), aflatoxin P1 (AFP_1), and free guanine residues, AFB_1-N_7 -guanine in urine, while rats excrete AFB_1 in the intestinal tract through bile. Ruminants excrete AFB_1 mainly through faeces and AFM_1 predominantly through urine and milk.

Aflatoxin contamination of animal foods

Besides the hepatotoxic and immunosuppressive nature of aflatoxin, it is a group-1 carcinogen that triggers stringent regulations not only on agricultural commodities but also on animal products. The quantity of aflatoxin M_1 in dairy products and aflatoxin B_1 in eggs and meat products is dependent on the contamination level of feeds offered to food animals. Aflatoxin M_1 has been detected in human breast milk and lactating animals exposed to aflatoxin contaminated foods. The carry over rate of aflatoxin M_1 varies from 0.8-6.5% depending on the species, milk yield and stage of lactation (Churchill, 2017). Although certain levels of reduction in the concentration of aflatoxin M_1 have been reported in pasteurized ultra-high temperature (UHT) milk and milk products than the raw milk, it is known to be stable under various processing conditions (Table 2). In a study, Iqbal *et al.* (2014) reported contamination of AFB-1 in 35% of samples of broiler chicken meat and 28% sample of eggs from Punjab province of Pakistan. The higher concentrations of aflatoxins were also reported in sausages and other meat products in many parts of the globe (Aziz and Youssef, 1991; Shaltout *et al.*, 2014). As the consumption of animal products increases, the presence of aflatoxins in milk, meat and eggs is alarming for the health of human beings.

Effects of aflatoxins on livestock health

Mycotoxins are deadly cytotoxic; disintegrate cellular structures such as membranes (Alizadeh *et al.*, 2021). Mycotoxins interfere with vital cellular processes such as translation, transcription and replication machinery of cell synthesis. They have adverse effects on humans, livestock and crops that result in illnesses and serious economic losses (Meyer *et al.*, 2016). Mycotoxins come in the animals or humans through contaminated feeds/ food infested with spores, conidiospores and/or fragments of mycelium (foodborne zoonosis impacts). Aflatoxins are detoxified by ruminants through the liver and excreted in the bile juice. Thus, mycotoxins can be classified as hepatotoxins, nephrotoxins, neurotoxins and immunotoxins (Ráduly *et al.*, 2020). Cell biologists put them into generic groups such as teratogens, mutagens, carcinogens, and allergens. Aflatoxin, especially aflatoxins B_1, is a potent hepatotoxic and carcinogenic agent. The major health effects of aflatoxicosis in various species have been described in Table 3.

Aflatoxins create both acute and chronic impacts in livestock and produce various deleterious effects such as acute liver damage, liver cirrhosis, induction of tumours and teratogenic effects (Bennett and Klich, 2003). Aflatoxicosis is mainly a hepatic disease. The susceptibility of individual animals towards aflatoxins varies considerably and depends on species, age, sex, and nutrition. In fact, aflatoxins are hepatotoxic and compromise milk and egg production. Its recurrent infection occurs as a result of immunity suppression (Bennett and Klich, 2003). All age groups are susceptible to aflatoxicosis, but still, young species are most susceptible. Clinical signs of aflatoxicosis in animals include gastrointestinal dysfunction, suppressed fertility, reduced feed utilization and efficiency, anaemia and jaundice. Suckling animals may be affected as a result of the conversion of aflatoxin B_1 to the metabolite aflatoxin M_1 excreted in the milk of dairy animals (Pier *et al.*, 1980; Robens and Richard, 1992). Domestic animals, monkeys and laboratory rats, guinea pigs and mice have been the subject of a large body of research on the adverse effects of aflatoxins (mainly B_1). These effects include adducts and mutations, cancer, immunosuppression, lung injury and birth defects. Also, aflatoxins have been shown to interact with DNA (nuclear and mitochondrial adducts) and polymerases responsible for DNA and RNA synthesis (Bennett and Klich, 2003). Among suckling calves, acute aflatoxicosis develops a disease that features blindness, circling and falling down, twitching of ears and grinding of teeth. Spasm of the rectum is seen in most cases. Death usually follows within two days of the onset of severe clinical signs. Postmortem findings of aflatoxicosis reveal pale, firm and fibrosed liver. The kidneys are yellow and surrounded by wet fat (Pier *et al.*, 1980; Diekman and Green, 1992). Other pathological features in cattle are impaired blood coagulation due to prothrombin, factors

Table 2. Aflatoxin levels in various animal products of India and neighbouring countries

Animal products	Region	Aflatoxin type	No. of positive samples	AFM_1 level (mean/range)	References
Liquid cow milk	UP, India	AFM_1	4 (12)[1]	2.8–16.4 μg/kg	Rastogi *et al.*, 2004
Raw cow milk	Karnataka and Tamilnadu, India	AFM_1	45 (45)[1]	0.1-3.8 μg/kg	Siddappa *et al.*, 2012
Ultra-high temperature (UHT) cow milk	Karnataka and Tamilnadu, India	AFM_1	29 (45)[1]	0.06-2.1 μg/kg	Siddappa *et al.*, 2012
Infant milk food	UP, India	AFM_1	17 (17)[1]	7.7-8.44 μg/kg	Rastogi *et al.*, 2004
Buffalo milk	Maharashtra, India	AFM_1	16 % [1]	0.026 μg L^{-1}	Nile *et al.*, 2016
Cow milk	Maharashtra, India	AFM_1	44% [1]	0.018 μg L^{-1}	Nile *et al.*, 2016
Goat milk	Maharashtra, India	AFM_1	10 % [1]	0.014 μg L^{-1}	Nile *et al.*, 2016
Yogurt	Bangladesh	AFM_1	-	0.83-4.11 μg/L	Sumon *et al.*, 2021
Milk powder	Bangladesh	AFM_1	-	0.59-0.7 μg/L	Sumon *et al.*, 2021
Butter	Pakistan	AFM_1	52% [1]	6.97 μg/kg	Iqbal and Asi, 2013
Farm eggs	Pakistan	AFB_1	-	2.41 μg/kg	Iqbal *et al.*, 2014
Chicken broiler (Chest)	Pakistan	AFB_1	-	1.19 μg/kg	Iqbal *et al.*, 2014
Chicken broiler (Liver)	Pakistan	AFB_1	-	2.64 μg/kg	Iqbal *et al.*, 2014
Meat sausage	Egypt	AFB_1	-	9.03 μg/kg	Shaltout *et al.*, 2014
Luncheon meat	Egypt	AFB_1		8.8 μg/kg	Shaltout *et al.*, 2014

[1] Exceeding European Community (EC)/ Codex regulations (0.05 μg/kg)

VII and X and possibly factor IX. A single small dose of aflatoxin causes mounting of the plasma enzymes levels of aspartate aminotransferase, lactate dehydrogenase, glutamate dehydrogenase, gamma-glutamyltransferase and alkaline phosphatase and concentration of bilirubin, probably reflecting liver damage. Other abnormal clinical findings of aflatoxicosis are proteinuria, ketonuria, glycosuria and haematuria (Hussein and Brasel, 2001).

The carcinogenic effects of aflatoxins have been extensively studied. Aflatoxin B_1, aflatoxin M_1 and aflatoxin G_1 have been shown to cause various types of cancer in different animal species. However, only aflatoxin B_1 is considered as carcinogenic in experimental animals (Bennett and Klich, 2003).

Prevention and amelioration

There are various strategies for the prevention of aflatoxin contamination in feeds as well as treatment and preventive antidotes. A strategy to control aflatoxin concentration in feeds would require an understanding of the etiology of aflatoxin production and the ecology that supports it in pre-and post-harvest circumstances (Magan and Aldred, 2007). The extent and nature of aflatoxin contamination in feeds should be extensively studied through the collection and interpolation of surveillance data. Based on scientific studies on aflatoxins, legislation imposing limits to their concentration in foods and feeds has been set up, which should be under regular overview and scrutiny. Even with good manufacturing practices, aflatoxin is

Table 3. Effects of aflatoxins on health of various animal species

Species	Effects	References
Human	Hepatocellular carcinoma, pulmonary oedema, fatty infiltration and liver necrosis, vomiting, abdominal pain, growth impairment, acute hepatic encephalopathy in children, bile duct proliferation	Kensler *et al.*, 2011; Wu *et al.*, 2014
Cattle	Liver cirrhosis, bile duct proliferation, periportal fibrosis, jaundice of the mucous membrane, wide spread haemorrhages, fatty accumulations in the liver, acute mastitis, rectal prolapse, cancer, birth defects, genetic alterations, small and unhealthy calves, diarrhoea, respiratory distress, hair loss	Applebaum *et al.*, 1982; Guthrie and Bedell, 1979
Sheep	Inappetence, apathy, neurological signs, gross and microscopic lesions confined to the liver, nasal and liver tumours	Suliman *et al.*, 1987; Lewis *et al.*, 1967
Poultry	Increased mortality in interaction with infectious bursal disease (IBD), impaired immune response, reduced body weight, feed conversion efficiency, average daily gain, feed conversion ratio, reduced egg production, egg weight and yolk weight, changes in yolk colour, shell weight and shell integrity	Smith and Hamilton, 1970; Huff *et al.*, 1986; Dersjant-Li *et al.*, 2003; Otim *et al.*, 2005; Zaghini *et al.*, 2005
Pig	Anorexia, nervous signs, sudden death, damaged white blood cells (loss of immunocompetence)	Kanora and Maes, 2010; Marin *et al.*, 2002
Rabbit	Teratogenicity, pneumonia, nephrosis, catarrhal enteritis, icteric and enlarged liver, degeneration of seminiferous tubule epithelium	Lakkawar *et al.*, 2004; Wangikar *et al.*, 2005
Aquatic species	Refusal of feed, jaundice, weight loss, reduction in feed efficiency, liver dysfunction, cellular damage	Santacroce *et al.*, 2008

considered as an unavoidable contaminant in feeds and foods. To undertake preventive and ameliorative measures for mould growth, it is very necessary to analyze the aflatoxin levels in feeds (Alvarado *et al*., 2017).

Importance of aflatoxin analytical procedures

To estimate the nature and extent of aflatoxin contamination in feeds, foods and raw agricultural products and to form basic quality control, processes of sampling, preparation of samples and analysis methods should be streamlined. A well-established pattern of sampling, extraction, clean-up, separation, detection, quantification, confirmation and risk assessment should be followed for analytical procedures to isolate aflatoxins. Coker *et al.* (1995) recommended single, composite samples of unprocessed kernels (e.g. corn, groundnuts, oil palm) to be within a range of 10-30 kg, constituted by about 100 incremental samples, while processed commodities (e.g. oilseed meals and cakes) should have samples of about 5 kg, constituted by about 50 incremental samples. Various diagnostic, analytical and immunological procedures like thin layer chromatography (TLC), enzyme linked immunosorbent assay (ELISA), liquid chromatography tandem mass spectrometry (LC-MS/MS), high performance liquid chromatography (HPLC) and electro-chemical immunosensors (ECI) are available in the present time for the estimation of aflatoxins in various feeds (Mushtaq *et al*., 2020). HPLC is a more advanced, precise and sensitive technique than TLC and ELISA.

Pre-harvest control of aflatoxin contamination

Prevention of aflatoxin production in agricultural products at the farm level involves improvement in management protocols like improvement in techniques of irrigation, addition of fertilizers, crop rotation, use of fungus resistant varieties of crops and pest control (Xu *et al*., 2021). Replacement of toxigenic strains of aflatoxins with competitive non-aflatoxin producing strains is another strategy. The non-toxigenic strains reside in niches similar to the natural toxigenic strains and are capable of competition and displacement of the toxigenic strains. Endogenous biological control of aflatoxin production through protective microbes called endophytes, which grow together with crops without any negative effect on their hosts, may help in reducing the seed mycotoxin contamination (Tsitsigiannis *et al*., 2012). One of the important avenues to explore is through increasing the ability of crops to provide resistance against fungal infection and prevention of aflatoxin production, which can be accomplished by genetic engineering and plant breeding of particular crops, although the process is labour-intensive and tedious (Fedoroff, 2010). There is a requirement for long-term sustainable and efficient strategies for pre-harvest interventions.

Post-harvest control of aflatoxin contamination

Among post-harvest interventions, the importance of proper storage conditions with the prevention of mechanical and insect damage, maintaining temperature, aeration and moisture are paramount. It directly influences toxin production and feed contamination by mould. Other than physical methods, the use of chemical preservatives (Singh *et al*., 2021) is also followed with use at internationally agreed levels. Sorbic acid and propionic acid are some examples of preservatives widely used on a commercial scale (Smith *et al*., 1994). It is of importance that adequate levels are used; otherwise inadequate levels may lead to increased aflatoxin levels in the products. Novel strategies include the use of gamma-irradiation, involving the use of very high-energy photons through a gamma-source like cobalt-60 resulting in damage to microbial cell DNA and destruction of pathogenic microbes (Markov *et al*., 2015). Various other strategies (Martinez *et al*., 2019; Gibellato *et al*., 2021) have been adopted for detoxification of aflatoxins in oilseeds and grains, such as food and feed processing, chemo-protection, dietary modifications, chemical degradation, bio-control and microbial inactivation and reduction of toxin bioavailability by selective chemisorption with clays (Smith *et al.*, 1994).

Treatment of aflatoxicosis

Specific treatment for aflatoxicosis has yet to be identified, so symptomatic treatment is recommended in case of the affected animals. Dietary manipulation can be followed, since a diet low in protein leads to increased aflatoxin susceptibility in animals, and supplementation with N-acetylcysteine, choline and methionine is beneficial (Seeff *et al.*, 2013). Vitamin E can be supplemented with the goal of reducing clinical expression of aflatoxicosis in mind, although it does not affect the severity or pathology of aflatoxicosis (Hope, 2013). The toxic effects can be reduced by the use of various materials, such as drugs and natural products to alter the process of aflatoxin biotransformation. Polyphenols present in green tea block or reduce the initiation of AFB_1-induced hepatocarcinogenesis by reducing the activity of CYP1A1 enzyme (Valencia-Quintana *et al.*, 2012). Products that adsorb and enterically bind aflatoxins, making them unavailable for absorption, have been studied. Hydrated sodium calcium aluminosilicate and other clays of the smectite group have been shown to decrease clinical expression of toxicity of dietary aflatoxins in many species (Attia *et al.*, 2019). Modified yeast cell wall (*Saccharomyces cerevisiae*) may also decrease absorption of aflatoxins in some species (Gonçalves *et al.*, 2017). However, the best prevention of aflatoxins is avoiding conditions suitable for aflatoxin formation and evading aflatoxin consumption.

Conclusion

In India and other developing countries, there are challenges to get aflatoxin free foods, as the climatic conditions are very favourable to mould growth. Therefore, systematic efforts are required to reduce aflatoxin exposure to animals through sustainable preventive strategies of pre and post-harvest management of food-feed crops. As the demand for animal products (meat, milk and eggs) is increasing globally, especially in developing countries, urgent steps are needed to monitor and control aflatoxin levels in animal products. Strict surveillance of animal products as well as feeds is necessary to avoid aflatoxin contamination and safe food production.

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Author's contribution: ST and RKS: Collected the information; AD, PSD, ST and RKS: Wrote the manuscript; AD: Critically reviewed and corrected the manuscript. All authors finally read and agreed to submit the manuscript.

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