Chapter 2
Mycotoxins and Their Consequences in Livestock

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2.1 Introduction

Mycotoxins are secondary metabolites of a fungus that produce toxic effects in another organism. These show high cytotoxic effect by disrupting cell structures such as membranes, and processes such as protein, DNA and RNA synthesis. They have low molecular weight (approximately 700 Da) but vary in structure and from species to species. These toxins could be present with destructive cytotoxicity in an organism even after removal of fungal pathogen. Thus, lack of visible appearance of fungus does not negate presence of mycotoxins. They are observed in cereal grains as well as nuts, coffee, cocoa, spices, oil seeds, dried peas, beans and fruit before, during and after harvest, in various environmental conditions (Abdel-Wahhab and Kholif 2008).

Mycotoxins are specific to temperate and tropical region based on the presence of fungi in the area. It has been estimated that about 25% of all crops worldwide are affected by moulds or fungi (Binder 2007) mainly during storage. The absence of mould or fungi is not a guarantee of mycotoxin-free crop as these can be present intact in a crop even after the producer fungi is dead. Mycotoxins may be heat stable and these cannot be destroyed by canning or other processes.

The term "mycotoxin" become highly important after death of thousands of turkeys in 1962 near London, England, due to the mysterious turkey X disease which was later linked to feeding of peanut (groundnut) contaminated with secondary metabolites of *Aspergillus flavus* (Pitt 2013). These mycotoxins called aflatoxins

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(AF) from *A. flavus* were the first mycotoxins to be identified in 1965. The well-known antibiotic penicillin is also a mycotoxin (Speight 2012).

Mycotoxin-producing moulds belong to the genera *Aspergillus, Penicillium* and *Fusarium*. Till date, there are about 200 fungal species known to produce more than 400 mycotoxins (Liew and Mohd-Redzwan 2018). Some of the most agriculturally important mycotoxins, i.e. aflatoxins, fumonisins, trichothecenes, ochratoxins, sterigmatocystins (STCs) and zearalenones (ZEAs), are the only few mycotoxins being regulated or tested routinely (Streit et al. 2013; Wu et al. 2014; Urusov et al. 2015; Anfossi et al. 2016) despite the regular discovery of new ones. In addition, some mycotoxins (named as masked mycotoxins, e.g. zearalenone–glycoside) cannot be identified in these routine testing because of their modification after conjugation with plant metabolites (Anfossi et al. 2016). These masked or hidden mycotoxins get freed from conjugated molecules in human or animal alimentary canal and have shown their toxic effects (Liew and Mohd-Redzwan 2018). Children also have variation in immunological and physiological sensitivity to neurotoxins including endocrine susceptibility. It makes them more vulnerable to the toxicity of mycotoxins (Raiola et al. 2015). The exposure of mycotoxins and their economic impact on human and animal health are a global concern compounded by a range of regulatory challenges which often vary among countries. The effects of mycotoxins for a specific dose are more on children due to their lower body weight as compared to adults.

Secondary metabolite production in fungi may be by one of many metabolic pathways, for instance *Trichothecenes*, which are produced by the terpene pathway and rubratoxin by the tricarboxylic acid pathway. Some like aflatoxins may be produced by more than one route, e.g. the polyketide or amino acid pathway. Some are produced by a combination of routes as in the case of cyclopiazonic acid. In the host body mycotoxins may be removed by either bioactivation or detoxification. Detoxification occurs via enzyme-mediated biotransformation. These enzymes are induced endogenous enzymes including CYP450 isoforms and other enzymes of the host cells or digestive microbial flora. At lower concentration of toxins, these enzymes can alter the toxin to make its elimination through urine, faeces and milk feasible. At higher concentrations, toxins strongly inhibit this type of induction and may become fixed in animal or human tissues. Some of their metabolites like metabolite of AFB1, i.e. AFBO and FB1, lead to carcinogenesis by direct binding to or alteration of DNA. The accumulation of mycotoxins and their metabolites can elicit oxidative stress and alterations in the mitochondrial number and function that further impose adverse effects on cell metabolism by DNA damage and genome instability, cell cycle arrest, changes of cell organization and morphology, apoptosis and cell death (Wen et al. 2016).

Mycotoxin-contaminated forage highly affects the feed intake by animal and makes them weaker. Additionally, the remaining residues of mycotoxins in edible animal products, milk, meat and offal, could show detrimental effects on human health. Thus, various international agencies like WHO, European Food Safety Authority (EFSA), International Programme on Chemical Safety (IPCS), Food and Drug Administration (USA), etc. have set maximum tolerance limits for these in

feeds and milk. Experimental testing indicated refusal of toxic dose at 4–5 ppm deoxynivalenol (a trichothecene) in feed in dogs (Beagles and Brittany spaniels) and 7.5 ppm in cats. Vomiting in dogs and cats at 8–10 mg/kg concentration range has been observed. Anecdotal incidences of vomiting and feed refusal (breeds not specified) were also seen at approximately 1 to 4 ppm vomitoxin concentrations (Talcott 2013).

Mycotoxins rarely cause death in animals but manifest minor chronic troubles. There are several genetic, environmental and physiological factors which may interact in the pathogenesis shaping metabolism and toxicity which on exposure to a specific mycotoxin make diagnosis and confirmation of disease a challenge. After worldwide awareness about mycotoxin-caused illnesses, more than 100 countries have adopted regulations (van Egmond et al. 2007). Potential mycotoxicoses are better contained by rigorous testing of animal feed for fungal contamination apart from a strict control of how the feed is cultivated, harvested and stored. In most cases where the plant material is mild to moderately contaminated, mycotoxins can be diluted or even eliminated by suitable methods. Upon ingestion of contaminated feed by animals, efforts of reducing the bioavailability of mycotoxins in the GI tracts through adsorbents may be made.

2.2 Classification of Mycotoxins

Mycotoxins have been classified differently by researchers or groups according to their profession. Clinicians group mycotoxins based on affected organ as hepatotoxins, nephrotoxins, neurotoxins, immunotoxins and so forth. Cell biologists mention them as teratogens, mutagens, carcinogens and allergens. Organic chemists classify them by their chemical structures (e.g. lactones, coumarins). Biochemists give importance to biosynthetic origins and classify them as polyketides, amino acid derived, etc. Physicians name them by the illnesses they cause, e.g. St. Anthony's fire, stachybotryotoxicosis. Mycologists categorize them as per name of fungus producing them, e.g. *Aspergillus* toxins, *Penicillium* toxins, etc.

At present none of these classifications is entirely satisfactory. For example, aflatoxin is a hepatotoxic, mutagenic, carcinogenic, difuran-containing, polyketide-derived *Aspergillus* toxin. Zearalenone is a *Fusarium* metabolite with potent estrogenic activity. In this chapter let us focus on mycotoxins associated with food grains and ultimately mycotoxins which cause disease to human beings and livestock. The epidemic infections among livestock and humans are due to contaminated grain crop and their mycotoxins such as ergot alkaloids, aflatoxins and trichothecene. Several other mycotoxins together with ochratoxins, fumonisins, zearalenone and phomopsins causes infection mainly in livestock, but association with human diseases remains approved by scientific proof. Some of the major mycotoxins are classified below (Bennett and Klich 2003).

2.2.1 *Aflatoxins*

The first mycotoxin, aflatoxin, was recognized as a problem in Turkeys' X disease in UK poultry industry, and after killing more than 100,000 birds, it raised the research and economical importance of mycotoxins. Aflatoxins are potentially carcinogenic, mutagenic, immunosuppressive agents of fungi released by *Aspergillus flavus, A. parasiticus, A. fumigatus* and/or *Penicillium islandicum* in dried food and groundnut meal. They are identified as suspected causes of liver cancer in human beings. Other clinical symptoms due to aflatoxin poisoning are jaundice, portal hypertension, rapidly developing ascites, etc. Annually, aflatoxins can destroy an estimated 25% or more of the world's food crops; to minimize this economic loss, it is very important to determine even very low concentration in foods and feedstuff (https://www.who.int/foodsafety/FSDigest_Aflatoxins_EN.pdf visited on 28/12/2019). Many analytical techniques for aflatoxin detection as well as quantification have been described ranging from simpler thin-layer chromatography (TLC) to mass spectroscopy to the very sensitive high-performance liquid chromatography (HPLC). In the last few decades, enzyme-linked immunosorbent assay (ELISA) and electrochemical immunosensors have also been used for this purpose. About 18 variants of aflatoxins have been detected in food grains. The most abundant form is B_1 and other major forms are aflatoxin B_2, G_1 and G_2, while M_1 and M_2 are less significant (Wacoo et al. 2014).

2.2.2 *Ochratoxins*

Ochratoxins (OTAs) investigated initially in northern Europe and Africa in cereals are produced mainly by *Penicillium viridicatum* or *Aspergillus ochraceus* in maize, peanuts, beans, etc. OTAs are recognized as severely nephrotoxic which may become embryotoxic, teratogenic, and immunotoxic on further higher doses (Reddy and Bhoola 2010). OTAs are detected routinely by various methods adopted by individual laboratories. The detection methods are conventional chromatographic methods, high-performance liquid chromatography (HPLC), liquid chromatography tandem mass spectrometry (LC-MS/MS), affinity probe capillary electrophoresis (APCE) assay, fluorescein-tagged OTA aptamer (F-Apt-O)-based assays, immunoassays, enzyme-linked immunosorbent assays (ELISA), chemiluminescent ELISA and other OTA-specific DNA aptamer-related techniques. Aptamer-related techniques are comparatively newer detection methods with high sensitivity and accuracy (Ha 2015).

2.2.3 Zearalenone

Members of *Fusarium* such as *F. moniliforme* and *F. graminearum* produce zearalenone, a phenolic resorcylic acid lactone in maize and other cereals. Symptoms associated with zearalenone (ZEN) were first reported in the 1960s by two separate groups of investigators in livestock feed in swine in Minnesota, USA (Christensen et al. 1965). In 1963, herds of young swine in Minnesota had symptoms which included tumefaction of the vulva, prolapsed vagina and hypertrophy of the mammary glands. The feed was suspected for these symptoms and was given to guinea pigs and white rats which developed enlarged uteri, thus confirming the contamination in feed. The next year (1964), another group fed a swine herd with grain containing 30% mould-ridden corn and 70% sound corn and reported the development of similar symptoms. Mouldy corn contains two compounds which were initially coded as F1 and F2 (Christensen 1963). Of these, F1 tested as ergosterol (Christensen et al. 1965) and F2 as zearalenone (Urry 1966). Urry (1966) named F2 as zearalenone (ZEN) based on its structure and name of the producer fungus (*Fusarium graminearum*; teleomorph *Gibberella zeae*).

2.2.4 Trichothecenes

These are field toxins instead of storage toxins. Trichothecenes is a group of mycotoxins produced by members of genera *Fusarium, Myrothecium, Acremonium* (*Cephalosporium*), *Cylindrocarpon*, *Dendrodochium*, *Myrothecium*, *Trichoderma*, *Trichothecium* and *Stachybotrys* (Rai and Varma 2010; Bottalico and Perrone 2002). These mycotoxins can be identified in cereal crops such as wheat, barley, oats, rye, maize and rice (Yazar and Omurtag 2008) and other crops such as soybeans, potatoes, sunflower seeds, peanuts and bananas. These survive even processing of contaminated cereals for food preparation. Thus, it has been identified in bread, breakfast cereals, noodles and beer too (Bennett and Klich 2003). The mycotoxins are further classified into type A trichothecenes mainly including T-2, HT-2 and DAS and type B trichothecenes including DON, NIV, 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol (Ueno et al. 1975).

Trichothecenes have toxic effects on both humans and animals, manifested by subepidermal haemorrhage, several local irritations and cell or tissue necrosis. They are also responsible for anorexia, nausea, vomiting, gastroenteritis and haematological disorders (Pestka and Casale 1990). All trichothecenes attack the 60S ribosomal subunit, exhibiting toxicity through translational inhibition. They also inhibit synthesis of protein, RNA and DNA as well as mitochondrial and electron chain function. Inhibition of protein synthesis occurs through interference with peptidyl transferase activity. Trichothecenes can also stimulate lipid peroxidation, alter cell membrane function and neurotransmitter levels, induce

apoptosis and gene expression of numerous chemokines and cytokines, modulate immune responses as well as activate mitogen-activated protein kinases (MAPKs).

2.3 Factors Affecting the Growth of Mycotoxins

Based on growth conditions, there are three groups of factors – physical, chemical and biological – responsible for growth regulation of mycotoxins. These are discussed in some detail below.

2.3.1 Physical Factors

The locations of cereals or food are either field or storage house (cold stores, warehouse). The moisture and temperature as well as light intensity at these sites directly regulates the growth of fungi and ultimately mycotoxins.

2.3.1.1 Moisture

Moisture is involved in spore germination and growth of fungi. Saprophytic and decay fungi growing in the field require a higher moisture level in the substrate (22–25% wet weight) than those found in storage, if other factors are considered constant. Storage fungi can grow on substrates with 13–18% moisture level. Hesseltine (1976) experimentally suggested an optimal moisture level for aflatoxin formation in solid substrates. They were also germinated spores of rust fungi and *Helminthosporium* in distilled water.

2.3.1.2 Temperature

Sorenson et al. (1967) studied the effect of temperature on aflatoxin formation in rice and showed that it could be produced at about 11 °C to slightly above 36°C. Under these conditions aflatoxin G1 formation does not exactly parallel aflatoxin B formation. Although *Aspergillus* grows at temperatures ranging from 6–8 °C up to 44–45 °C; however at temperatures above 37 °C, no aflatoxin is formed.

2.3.2 Chemical Factors

2.3.2.1 CO_2

Sanders et al. (1968) studied the effect of CO_2 on peanuts sprayed with spores of a known aflatoxin-producing strain of *A. flavus* at varied relative humidity and CO_2 levels but constant temperature. The production of aflatoxin was greatly reduced at high CO_2 level and low relative humidity. At 60% CO_2, no visible growth or sporulation was observed at 86% relative humidity while fungal spore and hyphae were abundant in air at this humidity.

2.3.2.2 Oxygen

All mycotoxin-producing fungi can grow in an environment having 80% CO_2 and 20% O_2 (Taniwaki et al. 2010). A decrease in oxygen level affects growth and development of fungal hyphae and spore germination. However, the lack of O_2 does not mean that mycelia or spores are killed. A reduction of oxygen level while controlling other conditions like pH, temperature and moisture can degrade the aflatoxin. Thus, nuts can be preserved longer during storage by reducing O_2 level (Scussel et al. 2011).

2.3.2.3 Mineral Nutrition

Similar to other eukaryotic living beings, fungi need a number of trace elements such as iron and zinc in their nutrition. Therefore, it is considered that these and other trace elements are essentially needed by fungi for mycotoxin production. Steele et al. (1973) investigated the optimum presence of 0.055–2.2 mg/1 zinc, 0.004–0.04 mg/1 copper and 1.2–24 mg/1 iron for the production of ochratoxin A by *Aspergillus ochraceus* NRRL 3174. Removing or reducing any of these elements resulted in poor growth and no ochratoxin formation.

2.3.3 Biological Factors

2.3.3.1 Plant Stress

Plant physiologist believes that plants under stress are highly vulnerable to fungal infection. APRES (1981) studied the plants grown under drought conditions for formation of aflatoxin in peanuts. Peanut plants were grown in two different cities and planted in adjacent plots, one irrigated and one non-irrigated. They were harvested at three different times. It was observed that level of aflatoxin and *A. flavus* was not proportionate to each other, but aflatoxin level was directly dependent on

the *A. flavus* activity, regulated by physical and chemical conditions. Overmature or damaged peanuts were prone to aflatoxin contamination.

2.3.3.2 Transmission of Mycotoxins

Mycotoxins can be transmitted from contaminated food (meat, eggs, milk, dairy products) and crops to animals or humans. Crops may be contaminated in the field or during storage, and if the same are utilized for cooking bread and other grain-based products, then these will also contain mycotoxins (Fig. 2.1).

Mycotoxin-producing fungi can grow on a wide range of crops/feed including cereal, grain, beans, peas, groundnuts and fruits. Fungal growth can also occur in the field or during transport and storage. Each mycotoxin-producing fungal species can produce various types of mycotoxins depending on climatic and environmental conditions. A number of mycotoxins can thus be present in a contaminated feed/crop. Individual mycotoxins rarely occur in isolation. This increases the toxicity.

Mycotoxins can invade seeds during storage or at the crop stage in the field. Sometime fungal growth can occur during storage and if they have already released mycotoxins, then one can observe a high load of mycotoxins before seeds are

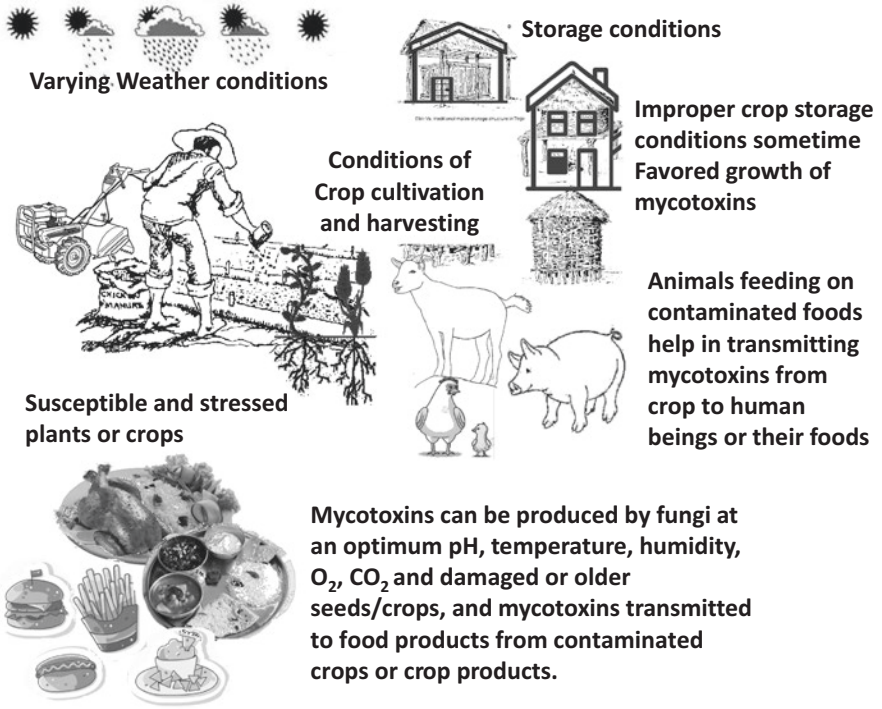

Fig. 2.1 Possible transmission route of mycotoxins from food chain to animals and human beings

received in feed mills or farms. Hence, preventing the occurrence of mycotoxins in feed ingredients can be a very difficult task. Non-homogeneous dispersion of mycotoxins in livestock feed makes it difficult to analyse. They may even go undetected in assays with perfect sampling procedures (Binder 2007).

2.3.3.3 Effect of Mycotoxins on Livestock

Many mycotoxins are potential carcinogens or threat to immune system as well as organ systems (kidney/liver). Mycotoxins survive during food and beer/spirits and wine preparation if grain or grapes are contaminated. Livestock health is at risk due to the level and the duration of exposure by mycotoxins. Mycotoxins could also interfere with bacterial cultures required for various food-processing activities, e.g. yoghurt and cheese making.

2.4 Mycotoxins in Pigs

Pigs are very sensitive to mycotoxins. The level and extent of exposure to mycotoxin(s) in feed and the age or immunity of the pig determine the degree to which animals are affected. In general, pigs that are young and of breeding age are more susceptible.

Mycotoxin contamination exposure can reduce the performance in growing and breeding pigs. It affects their immunity and health status and sometimes becomes fatal. A long and high-dose exposure may cause irreversible tissue damage that will compromise performance long after mycotoxins have been removed from the feed (Bennett and Klich 2003).

Pig feed has been reported to be contaminated with mainly AFB1, OTA (Ochratoxins), FB1 + FB2 (Fumonisin), T-2 + HT-2, DON (trichothecenes) and ZEA (zearalenones). DON mycotoxins can be responsible for digestive problems including feed refusal, vomiting and lesions of the gastrointestinal tract including weaker immune response (Smith et al. 2012). Fumonisin B1 consumption by gestating sows showed considerable damage to foetuses in utero thus resulting to economical losses in farm. Zearalenone is also a risk for reproduction in sows (Kanora and Maes 2009).

2.4.1 Factors That Causes Mycotoxins to Enter a Normal Pig

(a) The growth of mycotoxin-producing fungi (*Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Claviceps* spp.) can occur at various stages during the production process of animals and plants. Mould can grow during feed processing, especially when the mixer increases temperature and humidity in

the feed. Mould growth and mycotoxin production can also occur in insufficiently cleaned silos, transport systems and feeders at farm level. The fungi can contaminate various feed components like maize, wheat, barley, millet, peanuts, peas and oily feedstuffs. The production of mycotoxins is enhanced by factors like the humidity of the substrate (10–20%), the relative humidity (≥70%), the temperature (0–50 °C, depending on the fungal species) and the availability of oxygen (Kanora and Maes 2009).

(b) Mycotoxins may directly transmit to normal healthy pig from infected pig during sexual intercourse.

(c) The mycotoxin transmission is also associated with the health status of pig and other toxic entities.

2.4.2 *Mycotoxin Effect on Pigs and Its Associated Signs and Symptoms*

(a) *Aflatoxins* have shown carcinogenic, immunosuppressive, hepatotoxic and nephrotoxic effects on pigs.

(b) *Ochratoxin A* can cause decrease health performance (reduced weight gain), nephrotoxicity and gastrointestinal effects.

(c) *Ergot alkaloids* are responsible for reproductive defects and pathological complications. These effects can be observed by shrunken udders, stillbirths, reduced pregnancy rate, vasoconstriction in ear and necrosis of tails and hooves.

(d) *Zearalenone* also affects the reproductive system of male and female, manifested by disturbed cycle, conception nymphomania, feminization, impaired semen quality and testicular atrophy.

(e) *Deoxynivalenol (DON)* is associated with vomiting in swine and therefore sometime also known as vomitoxin.

2.5 Mycotoxins in Poultry

Mycotoxin infection in poultry may show a significant loss of meat productivity (Filazi et al. 2017) and poses a serious risk to human health. Poultry birds may metabolize mycotoxins in their alimentary canal, liver or kidneys in accordance with their chemical nature. Their metabolized derivatives then transfer to human beings through meat and eggs which leads to undesirable health effects. Toxicity of the mycotoxins and their derivatives depends on the amount of absorption, number of the metabolites formed, exposure period and sensitivity. Major mycotoxins which affect poultry species are aflatoxins (AF), ochratoxin A (OTA), fumonisins (FUM), deoxynivalenol (DON) and T-2 toxin.

2.5.1 Factors That Cause Mycotoxins to Enter in Healthy Poultry

The factors affecting poultry are similar to those affecting pigs. Environmental and biological factors including the breed and the offspring of infected species are responsible for ill effects of mycotoxins. The transmission routes of mycotoxins are ingestion of fungal spores, which are readily carried in the air or feed. High grain humidity and damage due to insects as well as poor storage conditions are major predisposing causes. Once toxins have been formed, it is difficult to avoid their biological effects as fungal spores and formed toxins are generally highly resistant. They also increase susceptibility to bacterial diseases.

2.5.2 The Effect of Major Mycotoxins on Poultry

2.5.2.1 Aflatoxins

They are usually found in feed ingredients used for poultry animals. Most extensive forms of aflatoxins (AF) include B1, B2, G1 and G2 (termed as AFB1, AFB2, AFG1 and AFG2, respectively). Among these AFB1 is most widespread and biologically active mycotoxin which causes decreased weight gain; poor feed efficiency; reduced egg production and egg weight; liver cirrhosis; reduced serum protein level; carcass bruising; poor pigmentation; reduced enzyme level and metabolism of starch, protein, lipids, and nucleic acids; and suppressed immune system. Domestic turkeys have been observed to be are significantly more susceptible to aflatoxin B1 compared to their wild counterparts. This has been linked to the loss of GST (glutathione S-transferases) alleles which afforded some resistance to the toxin through aflatoxin B1 protective glutathione S-transferases. This is just another instance of unintentional loss of helpful genes as a result of intensive breeding for traits useful for the poultry industry (Filazi et al. 2017). Murugesan et al. (2015) also reported a “remarkable loss” of rare alleles and single-nucleotide polymorphisms in commercial chicken breeds due to similar practices.

2.5.2.2 Ochratoxins

Among this group of mycotoxins, ochratoxin A (OTA) is the most prevalent in poultry animals. Toxicity symptoms include general weakness, anaemia and lower food consumption which translate into lower egg production and growth rates at lower dietary concentrations to excessive mortality at higher doses (Filazi et al. 2017). Pathophysiological studies showed a decrease in urine concentration and kidney performance, degeneration and ultrastructural alterations in integrity (Huff et al. 1975; Glahn et al. 1988, 1989). Murugesan et al. (2015) opined that there could be

an increase in relative weights of liver, spleen, pancreas, gizzard and testes if fed with OTA-contaminated foods.

2.5.2.3 Fumonisins

In comparison to horses and swine, two susceptible species, chicks and turkeys, are relatively resistant to the toxic effects of fumonisin (FB1). Mild to moderate toxicity was reported in chicks, ducks and turkeys. The primary effect of FB in chicks, ducks and turkeys is reduced rate of body weight gain and liver pathology. Chicks can show multifocal hepatic necrosis and biliary hyperplasia (Ledoux et al. 1992; Weibking et al. 1993), Hepatocellular hyperplasia and increased extramedullary hematopoiesis (Murugesan et al. 2015).

2.5.2.4 Trichothecenes

They show toxic effects as oral lesions, growth retardation, abnormal feathering, decreased egg production and egg shell quality, regression of the bursa of Fabricius, peroxidative changes in liver, abnormal blood coagulation, leucopoenia and proteinemia and immunosuppression. Dietary concentrations of 3–4 mg/kg of T-2 toxin leads to affected broiler performance where a lower dietary concentration (0.4 mg/kg) can reduce the performance of ducks (Murugesan et al. 2015; Pettersson 2012). T-2 concentrations for oral lesions are lower (0.4 mg/kg) than concentrations for weak chick performance.

2.5.3 Diagnosis

Mycotoxin infection should be diagnosed in a farm having a history of sign and symptoms of intoxication. A confirmation of intoxication involves detection and quantification of specific mycotoxins. In poultry a non-homogeneous mycotoxin dispersion and high volume of feeds and ingredients make diagnosis difficult. However, a simultaneous feed analysis and necropsy with related diagnostic tests can increase the chances of diagnosis. Frequent and multiple sampling from different sites may also increase the confirmation of a mycotoxin formation zone (hot spot). Samples should be collected at sites of ingredient storage, feed manufacture and transport, feed bins and feeders.

Concurrent diseases can adversely affect production and should be considered. Sometimes, mycotoxicosis is suspected but not confirmed by feed analysis. In these situations, a complete laboratory evaluation can exclude other significant diseases. Mycotoxin formation can be localized in a batch of feed or grain. Fungal activity increases as feed is moved from the feed mill to the feeder pans. Samples should be collected and submitted in separate containers. It should be noted that airtight

plastic/glass containers are not suitable for long-term storage only as they cause rapid deterioration of feed and grain.

2.5.4 Treatment

Removal of the source and causes of toxins is most effective treatment. Prevention can be managed by adding antifungal in feed preservatives. Feed with increased protein levels may also be beneficial. A mycotoxin-detoxifying agent dose of soluble vitamin A, C and E and selenium (0.2 ppm) in finely divided 1% copper sulphate for 7-day feed has been suggested; it can be given for 7 days wherever approved (Dvorska et al. 2007; Galvano et al. 2001).

2.6 Mycotoxins in Cattle

Dairy products are an important part of human diet and may pose a serious concern for human health if cattle are contaminated with mycotoxins since some mycotoxin metabolites like aflatoxin M1 may be excreted in milk (Whitlow and Hagler Jr 2005). Mycotoxins such as aflatoxins, fumonisins and trichothecenes and the occurrence of toxigenic fungi in feed and silage for dairy cattle may cause general, unspecific problems for animal production, such as reduced feed intake or feed acceptability. Mycotoxicoses may be recognized by large number of abrasions in organs including liver, kidneys, epithelial and central nervous system and various tissues according to the nature of particular mycotoxins. Thus, mycotoxicoses can cause significant losses to the animal industry worldwide (Goncalves et al. 2015).

2.6.1 The Effect of Major Mycotoxins in Cattle

Extremes in weather conditions may promote mycotoxin contamination of crops in the field, during harvest, or stored grains/cereals, with obvious detrimental results for cattle. A loss in crop yield is also observed if the spores in soil and/or water infect plants growing in the field. Fungal growth and the production of mycotoxins are proportional to each other. Mycotoxins majorly linked with cattle health are aflatoxin by *Aspergillus*; deoxynivalenol, zearalenone, T-2 toxin and fumonisin by *Fusarium*; and ochratoxin and PR toxin by *Penicillium*, and ergot mycotoxins may be common in certain feedstuffs (Whitlow and Hagler Jr 2005).

2.6.2 *Aflatoxins*

These are highly toxic, mutagenic and carcinogenic molecules in nature. Aflatoxin B1 is a carcinogen and excreted in milk in the form of aflatoxin M1. Cows consuming diets containing 30 ppb aflatoxin can produce milk containing aflatoxin residues above the FDA action level of 0.5 ppb. Acute aflatoxicosis in mammals has symptoms of inappetence, lethargy, ataxia, rough hair coat and pale, enlarged fatty livers. Chronic aflatoxicosis results in lower milk production, jaundice, loss of appetite, more susceptibility to diseases and obstruction with vaccine induced immunity in livestock. It may infect cattle through contaminated cereals, peanuts and cottonseed grown in warm and humid climates (Whitlow and Hagler Jr 2005).

2.6.3 *Deoxynivalenol*

This is the most commonly detected mycotoxin by *Fusarium* in feed. The impacts of DON on cattle are not well recognized. However, some clinical reports showed a relationship between DON contamination of diets and poor performance of cattle. It is also observed that pure DON added to diets does not have toxicity similar to feeds contaminated naturally with DON (Foster et al. 1986). DON family can be interrelated to symptoms that are different or more severe than expected.

2.6.4 *T-2 Toxin*

This occurs in a low proportion of feed samples and is associated with reduced feed consumption, loss in yield, gastroenteritis, intestinal haemorrhage, reduced reproductive performance and death. T-2 toxin is associated with gastroenteritis and intestinal haemorrhages. Weaver et al. (1980) showed that T-2 was associated with feed refusal and gastrointestinal lesions in a cow, but did not show a haemorrhagic syndrome. Cows exposed to T-2 may suffer from bloody diarrhoea, low feed intake, minimized milk production and absence of oestrus cycles (Kegl and Vanyi 1991). Serum immunoglobulins and complement proteins were lowered in calves receiving T-2 toxin (Mann et al. 1983).

2.6.5 *Fumonisin B1*

It was first isolated in 1988 from *Fusarium verticillioides*. Identified as a causative agent of leucoencephalomalacia in horses, pulmonary oedema in swine and hepatotoxicity in rats, it is thought to be a carcinogen and considered as a promoter

of oesophageal cancer in humans (Marasas 1995). Fumonisins are structurally similar to sphingosine and at high concentrations are toxic to myelin sheath and other parts of nerve tissues. These can block sphingolipid biosynthesis and therefore results in deterioration of concerned tissues. Fuminosins have been reported as potent toxins to beef cattle, dairy cattle and other ruminants (Whitlow and Hagler Jr 2005).

2.6.6 Diagnosis and Treatment

Diagnosis of mycotoxin infection is possible only by a multifarious approach. The presence of mycotoxins is confirmed by detection in either feedstuffs or animal tissues with a combination of previous history of mycotoxicosis. Detection of more than one mycotoxin in feedstuffs may lead to different clinical signs as toxicological properties including lesions may differ from single mycotoxin in experimental animals. Concerted effects of mycotoxins on each other may enhance or minimize mutual effects. Immunosuppressive mycotoxins may cause secondary infection of viruses, bacteria, or other parasites which is more noticeable than the primary infection. Therefore immunosuppression by mycotoxin must be diagnosed carefully by thorough clinical examination, appropriate diagnostic testing and proper historical evaluation.

Feed grains should be tested at harvesting and storage facilities. Further, maintenance of clean and dry storage conditions including spray or mixing of acids (e.g. propionic acid) is suggested to control the growth of moulds, but this is effective only before formation of toxins. An already formed mycotoxin cannot be removed by adding acid additives. At present we still lack of any antidote for mycotoxins, though some mycotoxins like aflatoxin can be effectively removed from feedstuffs by absorption with the help of aluminosilicates. The traditional method of air exclusion in silage storage as reducing the time of storage of prepared feed is also an effective way of avoiding mycotoxin formation.

2.7 Prevention and Control

Instead of removing and treating a mycotoxin infection, it is suggested that preventive measure and avoiding or reducing the exposure time and dose is the better approach. Recognition of risk factors can be helpful in management of prevention. Mycotoxicoses cannot be completely treated with medical therapy after diagnosis, and mycotoxins may even survive after treatment.

Since mycotoxins can enter crops during harvesting, storage or preparation of food and may contaminate both animals and humans, the infection of fungus and their mycotoxins should be prevented at all the steps of crop cultivation, processing and animal rearing.

2.7.1 Primary Prevention

A disciplined plan of suitable preharvest, harvest and postharvest prevention of crop infection can minimize mycotoxin infection. Development of fungal-resistant plant varieties is as vital as preventing infection of crops in field. Additionally, a low humidity and moisture content of seeds after harvesting and during storage is a preventive method for mycotoxin infection. Cold storage should be used for commodities. Applying fungicides and preservatives against fungal growth as well as prevention of seed damage by insect infestation is also useful to control mycotoxins. Only approved insecticides in recommended dosage should be applied.

2.7.2 Secondary Prevention

This is inevitable if some fungi have entered the chain at early phase. The growth of existing fungi should be removed or stopped to prevent further damage and contamination. Suggested steps included re-drying the products to prevent growth of infested fungi, removal of damaged/contaminated seeds, inactivation or detoxification of mycotoxins and protection of stored products by reversing the conditions that favour fungal growth.

2.7.3 Tertiary Prevention

This level of prevention is implemented if the feed and crops are heavily infected by fungi. In such a situation, both of the above preventions are not effective since it is too late to completely stop toxic fungi and reduce their toxin formation. However, some measures should be taken to prevent the transfer of fungi and mycotoxins to other foods and environment. The general practice recommends a complete destruction of the contaminated products. After that a detoxification is recommended to minimize the level of contamination.

Ultimately if all the above steps have been missed or failed and the fungus enters the food chain, the only way to prevent further spread is isolating the infected animals from healthy ones. Mating between healthy and infected animals should also be prevented.

2.8 Legal Disposition

Food and Drug Administration (FDA) is a regulatory body for toxicity in food and food products. It has given directions to limit aflatoxin to 20 parts per billion (ppb) in all foods and feed. It is a must for all agencies and manufacturers to test the food products at multilevel checkpoints during production. Testing agencies such as Grain Inspection Packers and Stockyards Administration must report the toxic levels of mycotoxins to the FDA. ELISA (enzyme-linked immunosorbent assay) can be used in testing as it is a cheaper and faster technique. The mean permissible level of mycotoxins in cow feed (whole-plant corn silage) to the total diet allowed by US Food and Drug Administration (FDA) or European Union can be calculated as [(mean concentration of mycotoxin detected × estimated daily silage intake)/(maximum permissible mycotoxin in diet allowed or stipulated by US FDA or European Union × total diet DMI)] × 100. Here DMI stands for dry matter intake (Ogunade et al. 2018).

2.9 Conclusion

Mycotoxin-producing fungi cause illness in humans and their livestock either directly or indirectly. Mycotoxicoses are the most common known diseases of fungi, and secondary metabolite toxins from *Aspergillus, Fusarium, Penicillium*, etc., are an important health hazard. Mycotoxins refer to any pharmacologically active metabolite responsible for vertebrate toxicity and have been classified into several chemically and source wise unrelated classes. These are formed in a strain-specific way and produce intricate and overlying toxigenic effects in susceptible organisms. They may cause carcinogenicity, inhibition of protein synthesis, immunosuppression, dermal irritation and other metabolic perturbations. Route of mycotoxin entry is usually ingestion of contaminated foods from crops or animals. Sometimes, inhalation of spores and direct dermal contact can also be reason of infection.

It is difficult to differentiate between mycotoxin infection and mycotoxicosis. Many times, moulds may be present without producing any toxin, and most of the time mycotoxins may remain after processing of food preparation. Thus, the demonstration of mould contamination is not the same thing as the demonstration of mycotoxin contamination. Nevertheless, there is enough data from various researches to conclude that mycotoxins pose a hazard to human and animal health, but simple detection of mycotoxins is not enough to show that they are the aetiological agents in a given veterinary or human health problem. Mycotoxicoses may thus be far more common than we think. It is easy to feature the symptoms of acute mycotoxin poisoning to other causes; the opposite is true of aetiology. Proving cancer and other chronic conditions as a result of exposure to mycotoxins is obviously difficult. In summary, in the lack of suitable analytical criteria and

confirmatory laboratory tests, mycotoxicoses will remain diagnostically scary diseases.

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