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ORIGINAL ARTICLE

Impact of mycotoxins on humans and animals

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Abstract Mycotoxins are secondary metabolites of molds that have adverse effects on humans, animals, and crops that result in illnesses and economic losses. The worldwide contamination of foods and feeds with mycotoxins is a significant problem. Aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins, and ergot alkaloids are the mycotoxins of greatest agro-economic importance. Some molds are capable of producing more than one mycotoxin and some mycotoxins are produced by more than one fungal species. Often more than one mycotoxin is found on a contaminated substrate. Mycotoxins occur more frequently in areas with a hot and humid climate, favourable for the growth of molds, they can also be found in temperate zones. Exposure to mycotoxins is mostly by ingestion, but also occurs by the dermal and inhalation routes. The diseases caused by exposure to mycotoxins are known as mycotoxicoses. However, mycotoxicoses often remain unrecognized by medical professionals, except when large numbers of people are involved. Factors influencing the presence of mycotoxins in foods or feeds include environmental conditions related to storage that can be controlled. Other extrinsic factors such as climate or intrinsic factors such as fungal strain specificity, strain variation, and instability of toxigenic properties are more difficult to control. Mycotoxins have various acute and chronic effects on humans and animals (especially monogastrics) depending on species and susceptibility of an animal within a species. Ruminants have, however, generally been more resistant to the adverse effects of mycotoxins. This is because the rumen microbiota is capable of degrading mycotoxins. The economic impact of mycotoxins include loss of human and animal life, increased health care and veterinary care costs, reduced livestock production, disposal of contaminated foods and feeds, and investment in research and applications to reduce severity of the mycotoxin problem. Although efforts have

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continued internationally to set guidelines to control mycotoxins, practical measures have not been adequately implemented.

1. Introduction

It is difficult to define mycotoxin in a few words. All mycotoxins are low-molecular-weight natural products (i.e., small molecules) produced as secondary metabolites by filamentous fungi. These metabolites constitute a toxigenically and chemically heterogeneous assemblage that are grouped together only because the members can cause disease and death in human beings and other vertebrates. Not surprisingly, many mycotoxins display overlapping toxicities to invertebrates, plants, and microorganisms (Bennett, 1987). The term mycotoxin was coined in 1962 in the aftermath of an unusual veterinary crisis near London, England, during which approximately 100,000 turkey poults died. When this mysterious turkey X disease was linked to a peanut (groundnut) meal contaminated with secondary metabolites from *Aspergillus flavus* (aflatoxins), it sensitized scientists to the possibility that other occult mold metabolites might be deadly (Bennett and Klich, 2003). While all mycotoxins are of fungal origin, not all toxic compounds produced by fungi are called mycotoxins. The target and the concentration of the metabolite are both important. Fungal products that are mainly toxic to bacteria (such as penicillin) are usually called antibiotics. Fungal products that are toxic to plants are called phytotoxins by plant pathologists. Mycotoxins are made by fungi and are toxic to vertebrates and other animal groups in low concentrations. Other low-molecular-weight fungal metabolites such as ethanol that are toxic only in high concentrations are not considered mycotoxins (Bennett, 1987).

Mycotoxins are a structurally diverse group of mostly small molecular weight compounds, produced mainly by the secondary metabolism of some filamentous fungi, or molds, which under suitable temperature and humidity conditions, and may develop on various foods and feeds, causing serious risks for human and animal health. Mycotoxins are secondary metabolites that have no biochemical significance in fungal growth and development; however, they vary from simple C4 compounds, e.g., moniliformin, to complex substances such as the phomopsins (Dinis et al., 2007). Currently, more than 300 mycotoxins are known, scientific attention is focused mainly on those that have proven to be carcinogenic and/or toxic. Human exposure to mycotoxins may result from consumption of plant-derived foods that are contaminated with toxins, the carry-over of mycotoxins and their metabolites in animal products such as meat and eggs (CAST, 2003) or exposure to air and dust containing toxins (Jarvis, 2002).

Toxigenic molds are known to produce one or more of these toxic secondary metabolites. It is well established that not all molds are toxigenic and not all secondary metabolites from molds are toxic. Examples of mycotoxins of greatest public health and agro-economic significance include aflatoxins (AF), ochratoxins (OT), trichothecenes, zearalenone (ZEN), fumonisins (F), tremorgenic toxins, and ergot alkaloids. These toxins account for millions of dollars annually in losses worldwide in human health, animal health, and condemned agricultural products. Factors contributing to the presence or production of mycotoxins in foods or feeds include storage, environmental, and ecological conditions. Often times most factors are beyond human control (Hussein and Brasel, 2001). Ochratoxin A (OTA) is a secondary metabolite produced by several species of *Aspergillus* and *Penicillium*. The toxin, which is a nephrotoxic and nephrocarcinogenic compound, has mainly been found in cereals as well as in other products like coffee, wine, dried fruits, beer and grape juice. It occurs in the kidney, liver and blood of farm animals by transfer from animal feed. Although its genotoxic power has so far not been definitively established, zearalenone (ZEA), produced by various species of *Fusarium*, in particular *Fusarium graminearum* and *Fusarium culmorum*, has an osteogenous action and is significantly toxic to the reproductive system of animals (Milicevic et al., 2010).

Human food can be contaminated with mycotoxins at various stages in the food chain (Bennett and Klich, 2003) and the most important genera of mycotoxigenic fungi are *Aspergillus*, *Alternaria*, *Claviceps*, *Fusarium*, *Penicillium* and *Stachybotrys*. The principal classes of mycotoxins include a metabolite of *A. flavus* and *Aspergillus parasiticus*, aflatoxin B_1 (AFB_1), the most potent hepatocarcinogenic substance known, which has been recently proven to also be genotoxic. In dairy cattle, another problem arises from the transformation of AFB_1 and AFB_2 into hydroxylated metabolites, aflatoxin M_1 and M_2 (AFM_1 and AFM_2), which are found in milk and milk products obtained from livestock that have ingested contaminated feed (Boudra et al., 2007). In 1993, the WHO-International Agency for Research on Cancer (WHO-IARC, 1993a,b) evaluated the carcinogenic potential of AF, OT, trichothecenes, ZEN, and F. Naturally occurring AF were classified as carcinogenic to humans (Group 1) while OT and F were classified as possible carcinogens (Group 2B). Trichothecenes and ZEN, however, were not classified as human carcinogens (Group 3). The health hazards of mycotoxins to humans or animals have been reviewed extensively in recent years (Yaling et al., 2008; Averkieva, 2009).

Mycotoxins are not only hard to define, they are also challenging to classify. Due to their diverse chemical structures and biosynthetic origins, their myriad biological effects, and their production by a wide number of different fungal species, classification schemes tend to reflect the training of the person doing the categorizing. Clinicians often arrange them by the organ they affect. Thus, mycotoxins can be classified as hepatotoxins, nephrotoxins, neurotoxins, immunotoxins, and so forth. Cell biologists put them into generic groups such as teratogens, mutagens, carcinogens, and allergens. Organic chemists have attempted to classify them by their chemical structures (e.g., lactones, coumarins); biochemists according to their biosynthetic origins (polyketides, amino acid-derived, etc.); physicians by the illnesses they cause (e.g., St. Anthony's fire, stachybotryotoxicosis), and mycologists by the fungi that produce them (e.g., *Aspergillus* toxins, *Penicillium* toxins). None of these classifications is entirely satisfactory (Bennett and Klich, 2003).

2. Occurrence and significance of mycotoxins in foods and feeds

Mycotoxicoses in humans or animals are characterized as food or feed related, non-contagious, non-transferable, non-infec-

tious, and non-traceable to microorganisms other than fungi. Clinical symptoms usually subside upon removal of contaminated food or feed. A wide range of commodities can be contaminated with mycotoxins both pre- and post-harvest (CAST, 2003). Aflatoxins (AFTs) are found in maize and peanuts, as well as in tree nuts and dried fruits. OTA is found mainly in cereals, but significant levels of contamination may also occur in wine, coffee, spices and dried fruits. Other products of concern are beans, roasted coffee and cocoa, malt and beer, bread and bakery products, wines and grape juices, spices, poultry meat and kidneys, pig kidneys and pork sausages (Milicevic et al., 2008).

2.1. Aflatoxins

The aflatoxins were isolated and characterized after the death of more than 100,000 turkey poults (turkey X disease) was traced to the consumption of a mold-contaminated peanut meal. The major aflatoxins are called B_1, B_2, G_1, and G_2 (based on their fluorescence under UV light (blue or green) and relative chromatographic mobility during thin-layer chromatography) M_1 and M_2 (produced in milk and dairy products) (Fig. 1) (D'Mello and MacDonald, 1997). Aflatoxin B_1 is the most potent natural carcinogen known and is usually the major aflatoxin produced by toxigenic strains (Squire, 1981). Aflatoxins are difuranocoumarin derivatives produced by a polyketide pathway by many strains of *A. flavus* and *A. parasiticus*; in particular, *A. flavus* is a common contaminant in agriculture. *Aspergillus bombycis, Aspergillus ochraceoroseus*, *Aspergillus nomius*, and *Aspergillus pseudotamari* are also aflatoxin-producing species, but they are encountered less frequently (Peterson et al., 2001).

Aflatoxin contamination has been linked to increased mortality in farm animals and thus significantly lowers the value of grains as an animal feed and as an export commodity. Milk products can also serve as an indirect source of aflatoxin. When cows consume aflatoxin-contaminated feeds, they metabolically biotransform aflatoxin B_1 into a hydroxylated form called aflatoxin M_1 (Van Egmond, 1989). Aflatoxin is associated with both toxicity and carcinogenicity in human and animal populations. The diseases caused by aflatoxin consumption are loosely called aflatoxicoses. Acute aflatoxicosis results in death; chronic aflatoxicosis results in cancer, immune suppression, and other "slow" pathological conditions. The liver is the primary target organ, with liver damage occurring when poultry, fish, rodents, and nonhuman primates are fed aflatoxin B_1. There are substantial differences in species susceptibility. Moreover, within a given species, the magnitude of the response is influenced by age, sex, weight, diet, exposure to infectious agents, and the presence of other mycotoxins and pharmacologically active substances. Thousands of studies on aflatoxin toxicity have been conducted, mostly concerning laboratory models or agriculturally important species (Cullen and Newberne, 1994).

Finally, it should be mentioned that *Aspergillus oryzae* and *Aspergillus sojae*, species that are widely used in Asian food fermentations such as soy sauce, miso, and sake, are closely related to the aflatoxigenic species *A. flavus* and *A. parasiticus*. Although these food fungi have never been shown to produce aflatoxin, they contain homologues of several aflatoxin biosynthesis pathway genes. Deletions and other genetic defects have led to silencing of the aflatoxin pathway in both *A. oryzae* and *A. sojae* (Takahashi et al., 2002).

2.2. Ochratoxins

Ochratoxin A (OTA) (Fig. 2) is produced by fungi of the genera *Aspergillus* and *Penicillium*. The major species implicated in OTA production includes *Aspergillus ochraceus*, *Aspergillus carbonarius*, *Aspergillus melleus*, *Aspergillus sclerotiorum*, *Aspergillus sulphureus*, *Pichia verrucossum*. However, *Aspergillus niger* and *Pichia purpurescens* are less important OTA producers (Benford et al., 2001). OTA is a frequent natural contaminant of many foodstuffs such as cocoa beans, coffee beans, cassava flour, cereals, fish, peanuts, dried fruits, wine, poultry eggs and milk (Weidenborner, 2001). The mycotoxin was reported in 35% in "under-five clinics" of breast milks in Southern province of Sierra Leone with up to 22% cooccurrence with aflatoxins. However, the scientists observed that whenever OTA was detected in high levels, AFB_1 was absent or present at very

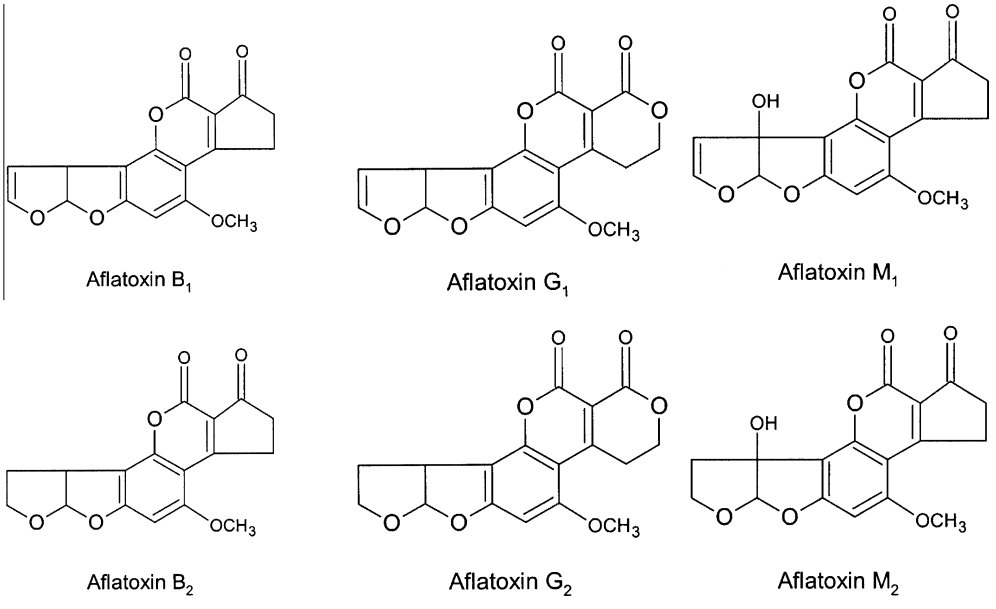

Figure 1 Chemical structure of aflatoxin B (AFB_1 and AFB_2), aflatoxin G (AFG_1 and AFG_2), and aflatoxin M (AFM_1 and AFM_2).

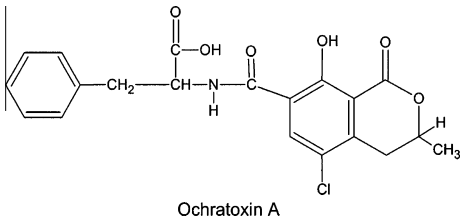

Figure 2 Chemical structure of ochratoxin A (OTA).

low levels and vice versa which suggests some sort of competition between these toxins either at the production level in foodstuffs or in their rate of absorption in the gastrointestinal tract. OTA has also been reported as a contaminant of tiger nuts and fermented maize dough in West Africa (Kpodo, 1996).

2.3. Fumonisins

Fumonisins (B_1 and B_2) (Fig. 3) are cancer-promoting metabolites of *Fusarium proliferatum* and *Fusarium verticillioides* that have a long-chain hydrocarbon unit (similar to that of sphingosine and sphinganine) which plays a role in their toxicity. Fumonisin B_1 (FB_1) is the most toxic and has been shown to promote tumor in rats and cause equine leukoencephalomalacia and porcine pulmonary edema. The naturally co-occurring aminopentol isomers (formed by base hydrolysis of the ester-linked tricarballylic acid of FB_1) have been suggested to exert toxic effects due to their structural analogy to sphingoid bases (Humpf et al., 1998). Consumption of fumonisin (Fig. 3) has been associated with elevated human oesophageal cancer incidence in various parts of Africa, Central America, and Asia and among the black population in Charleston, South Carolina, USA. Because fumonisin B_1 reduces uptake of folate in different cell lines, fumonisin consumption has been implicated in neural tube defects in human babies. Some correlation studies have suggested a link between the consumption of maize

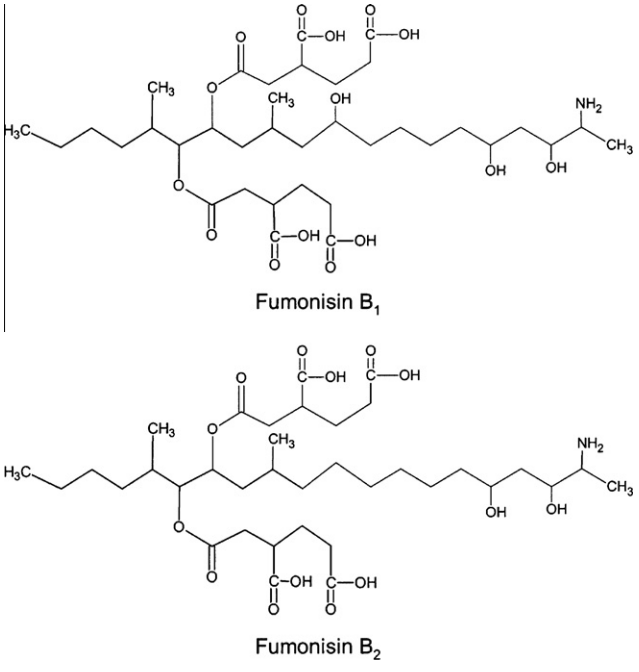

Figure 3 Chemical structure of fumonisins B_1 and B_2.

with high incidence of *F. verticillioides* and fumonisins and the high incidence of human oesophageal carcinoma in certain parts of South Africa (Marasas et al., 2004).

2.4. Trichothecenes

The trichothecene mycotoxins (TCT) (Fig. 4) comprise a vast group of over 100 fungal metabolites with the same basic structure. Several fungal genera are capable of producing TCT; however, most of them have been isolated from *Fusarium* spp. All trichothecene contain an epoxide at the $C_{12,13}$ positions, which is responsible for their toxicological activity. At the cellular level, the main toxic effect of TCT mycotoxins appears to be a primary inhibition of protein synthesis. TCT affect actively dividing cells such as those lining the gastrointestinal tract, the skin, lymphoid and erythroid cells. The toxic action of TCT results in extensive necrosis of the oral mucosa and skin in contact with the toxin, acute effect on the digestive tract and decreased bone marrow and immune function (Schwarzer, 2009). The trichothecene mycotoxins occur worldwide in grains and other commodities. Toxin production is greatest with high humidity and temperatures of 6–24 °C. Natural occurrence of TCT has been reported in Asia, Africa, South America, Europe, and North America (Scott, 1989). Trichothecenes have been detected in corn, wheat, barley, oats, rice, rye, vegetables, and other crops. They are common contaminants of poultry feeds and feedstuffs and their adverse effects on poultry health and productivity have been studied extensively (Leeson et al., 1995). Examples of type A TCT include T-2 toxin (T-2) and HT-2 toxin (HT-2), and diacetoxyscirpenol (DAS). Fusarenone-X (FUX), deoxynivalenol (DON), and nivalenol (NIV) are some of the common naturally occurring type B TCT. Types A and B trichothecene are distinguished by the presence or absence of a carbonyl group at the C8 position, respectively (Schwarzer, 2009).

Nivalenol was usually found associated with DON and its derivatives (mono-acetyldeoxynivalenols), along with FUX, which were produced by *F. graminearum, Fusarium cerealis, Fusarium culmorum* in the southern areas and in northern areas, by *Fusarium poae*. Moreover, from central to northern

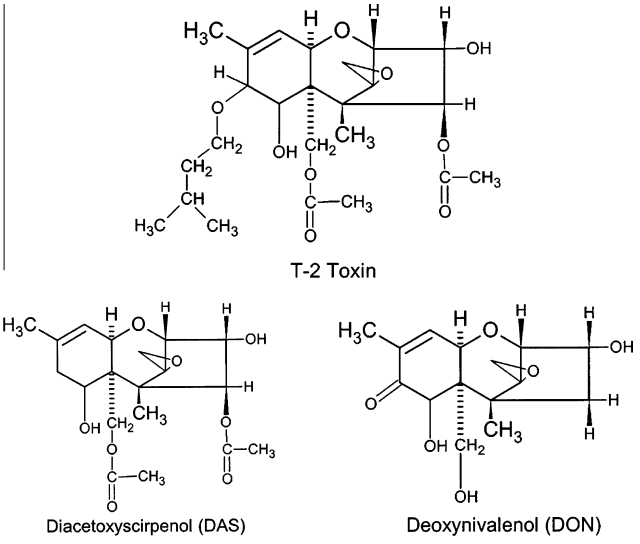

Figure 4 Chemical structure of T-2 toxin, diacetoxyscirpenol (DAS) and deoxynivalenol (DON).

European countries, moniliform has been consistently reported, as a consequence of the widespread distribution of *Fusarium avenaceum*, whereas the occurrence of T-2 derivatives, such as T-2 and HT-2, and DAS have been recorded in conjunction with sporadic epidemics *of Fusarium sporotrichioides* and *F. poae* (Bottalico and Perrone, 2002).

Rainbow trout and channel catfish trial data indicates the impact of T-2 toxin (up to 5 ppm) or Don (up to 15 ppm) diet supplementation on growth rate, feed efficiency, hematocrit, intestinal hemorrhaging (Manning et al., 2003).

2.5. Zearalenone

Zearalenone (Fig. 5) is a mycotoxin produced by *F. graminearum* and other *Fusarium* molds using corn, wheat, barley, oats and sorghum as substrates. It is a non-steroidal compound that exhibits oestrogen-like activity in certain farm animals such as cattle, sheep and pigs. Zearalenone is a phenolic resorcyclic acid lactone with potent oestrogenic properties, produced primarily by *Fusarium* (Schwarzer, 2009). Zearalenone is a phytoestrogenic compound known as 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcylic acid μ-lactone. It is a metabolite primarily associated with several *Fusarium* species (i.e. *F. culmorum*, *F. graminearum*, and *F. sporotrichioides*) with *F. graminearum* being the species most responsible for the oestrogenic effects commonly found in farm animals. Alcohol metabolites of ZEN (i.e. α-zearalenol and β-zearalenol) are also oestrogenic (Cheeke, 1998a).

2.6. Moniliformin

Moniliformin (i.e. a potassium or sodium salt of 1-hydroxycyclobut-1-ene-3,4-dione, Fig. 6) is produced by several *Fusarium* species (mainly *F. proliferatum*) and is usually found on the corn kernel. It can be transferred to next generation crops and survive for years in the soil. Although both FB_1 and moniliformin are produced by the same fungal species (*F. proliferatum*) no structural resemblance is found between the two toxins (Price et al., 1993).

3. Negative effects of mycotoxins on humans

Mycotoxicoses, like all toxicological syndromes, can be categorized as acute or chronic. Acute toxicity generally has a rapid onset and an obvious toxic response, while chronic toxicity is characterized by low-dose exposure over a long time period, resulting in cancers and other generally irreversible effects (James, 2005). Prior to the discovery and implementation of modern milling practices, *Fusarium* species have been implicated in several human outbreaks of mycotoxicoses. Cereal grains contaminated with *F. sporitrichoides* and *F. poae* were implicated in alimentary toxic aleukia in Russia from 1932 to 1947. Symptoms included mucous membrane hyperaemia, oesophageal pain, laryngitis, asphyxiation, gastroenteritis, and vertigo (Lewis et al., 2005).

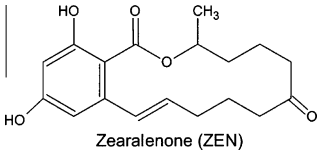

Figure 5 Chemical structure of zearalenone (ZEN).

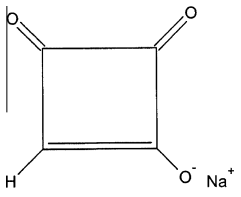

Figure 6 Chemical structure of moniliformin.

Aflatoxicosis is a toxic hepatitis leading to jaundice and, in severe cases, death. Repetitive incidents of this nature have occurred in Kenya (during 1981, 2001, 2004 and 2005), India, and Malaysia (Shephard, 2004; Lewis et al., 2005). AFB_1 has been extensively linked to human primary liver cancer in which it acts synergistically with HBV infection and was classified by the International Agency for Research on Cancer (IARC) as a human carcinogen (Group 1 carcinogen) (IARC, 1993). This combination represents a heavy cancer burden in developing countries. A recent comparison of the estimated population risk between Kenya and France highlighted the greater burden that can be placed on developing countries (Shephard, 2006).

The largest risk of AF to humans is usually the result of chronic dietary exposure. Such dietary AF exposures have been associated with human hepatocellular carcinomas, which may be compounded by hepatitis B virus. Approximately 250,000 deaths are caused by hepatocellular carcinomas in China and Sub-Saharan Africa annually and are attributed to risk factors such as high daily intake (1.4 μg) of AF and high incidence of hepatitis B (Wild et al., 1992). Aflatoxins have been found in tissues of children suffering from Kwashiorkor and Reye's syndrome and were thought to be a contributing factor to these diseases. Reye's syndrome, which is characterized by encephalopathy and visceral deterioration, results in liver and kidney enlargement and cerebral edema (Blunden et al., 1991). Aflatoxin has long been linked to Kwashiorkor, a disease usually considered a form of protein energy malnutrition, although some characteristics of the disease are known to be among the pathological effects caused by aflatoxins in animals. Aflatoxin exposure was associated with reduced levels of secretory immunoglobulin A (IgA) in Gambian children (Turner et al., 2003). Changes in differential subset distributions and functional alterations of specific lymphocyte subsets have been correlated with aflatoxin exposure in Ghanaian adults and indicate that aflatoxins could cause impairment of human cellular immunity that could decrease resistance to infections (Jiang et al., 2005).

Of the other health risk factors, the morbidity and mortality associated with unsafe sex, unsafe water and indoor smoke, arises from infectious diseases, such as HIV/AIDS, infectious diarrhoea and lower respiratory tract infection, respectively. The immunological suppression associated with aflatoxin and possibly DON could adversely affect all these outcomes. The modulating effect of aflatoxins in cases of zinc, iron and vitamin A deficiency in human health is less clear, but evidence from animal nutrition would suggest it could be significant (Williams et al., 2004). Fumonisins have been implicated in one incident of acute food-borne disease in India in which the occurrence of borborygmy, abdominal pain, and diarrhoea was associated with the consumption of maize and sorghum contaminated with high levels of fumonisins. Fumonisin B_1, the most abundant of the numerous fumonisin analogues,

was classified by the IARC as a Group 2B carcinogen (possibly carcinogenic in humans) (IARC, 2002). Fumonisins, which inhibit the uptake of folic acid via the folate receptor, have also been implicated in the high incidence of neural tube defects in rural populations known to consume contaminated maize, such as the former Transkei region of South Africa and areas of Northern China (Marasas et al., 2004).

Both DON and ZEN from toxic *Fusaria* have been linked to scabby grain toxicoses in the USA, China, Japan, and Australia. Symptoms included nausea, vomiting, and diarrhea. Fumonisin B_1 was associated with an illness outbreak in India with symptoms of acute onset of abdominal pain and diarrhea. Fumonisins also have been implicated in oesophageal cancer in China (Yoshizawa et al., 1994). However, with limited causal relationships and the presence of several confounding factors, data compiled by the International Agency for Research on Cancer were not conclusive for F carcinogenicity in humans (Casegnaro and Wild, 1995). Trichothecenes have been suggested as potential biological warfare agents. For example, T-2 toxin was implicated as the chemical agent of 'yellow rain' used against the Lao Peoples Democratic Republic from 1975 through 1981 (Peraica et al., 1999). In an investigation of similar biological warfare agents in Cambodia from 1978 to 1981, T-2 toxin, DON, ZEN, nivalenol, and DAS were isolated from water and leaf samples collected from the affected areas (Peraica et al., 1999).

Clinical symptoms preceding death included vomiting, diarrhea, hemorrhage, breathing difficulty, chest pain, blisters, headache, fatigue, and dizziness. In addition to nephritic congestion, autopsy findings included necrosis of the lining of the stomach and upper small intestine, lungs, and liver. It should be noted, however, that the origin of the samples of yellow rain is still a subject of debate. For example, one theory attributed the source of illnesses to unidentified endemic factors because the yellow rain was found to be a native bee fecal material devoid of mycotoxins (Seeley et al., 1985).

4. Negative effects of mycotoxins on non-ruminants

Early studies on the effects of acute aflatoxicosis indicated various toxicities in different animal species. In monogastrics, variable responses have been shown with all mycotoxins. For example, pigs have been shown to be very sensitive to T-2 toxin, DON, and ZEN. Poultry also are adversely affected by both T-2 and DON but are very resistant to the oestrogenic effects of ZEN (Cheeke, 1998a). Various degrees of mycotoxicoses from natural sources occur in different animal species because of the wide range of feed ingredients used and the differences among and within species. Experiments and case studies on mycotoxicoses in non-ruminant species have been summarized in the following sections (Hussein and Brasel, 2001).

4.1. Poultry

The negative effects of mycotoxins on chicken performance have been demonstrated in numerous studies. For example, feeding a high level (3.5 mg/kg of feed) of an AF mixture (i.e. 79% AFB_1, 16% AFG_1, 4% AFB_2, and 1% AFG_2) to broilers reduced their body weight and increased their liver and kidney weights (Smith et al., 1992). Aflatoxins also increased blood urea-N and decreased serum levels of total protein, albumin, triglycerides, and phosphorus. Feeding OTA (0.3–1 mg/kg of feed) to broilers reduced glycogenolysis and resulted in a dose-dependent glycogen accumulation in the liver. These negative metabolic responses were attributed to inhibition of cyclic adenosine 3′,5′-monophosphate-dependent protein kinase and were reflected in decreased efficiency of feed utilization and teratogenic malformations (Bitay et al., 1979). The activities of other enzymes (e.g. alkaline phosphatase, acid phosphatase, lactate dehydrogenase, and succinate dehydrogenase) in several organs (e.g. heart, liver, spleen, and pancreas) of 1-week-old chicks also were altered by ingesting feed contaminated with *Fusarium roseum*. Such change in enzyme activity resulted in metabolic and cellular respiratory disorders, reduced body weight gain, and tissue necrosis (Beri et al., 1991).

Fusarium mycotoxins have been shown to adversely affect poultry. In addition to reduced feed intake and body weight gain, buccal-oral ulceration and plaque formation were observed when 7-day-old chicks were given T-2 toxin (4 or 16 mg/kg of feed) or DAS (4 or 16 mg/kg of feed). Similar effects were also observed in 1-day–3-week-old chicks consuming T-2 toxin at 6 mg/kg of feed and in 24–25-week-old hens consuming DAS at 20 mg/kg of feed. Interestingly, fertility was increased in hens (67–69-week-old) and decreased in roosters (25–27-week-old) when DAS was fed at $\leqslant$5 and 10 mg/kg of feed, respectively (Brake et al., 2000).

4.2. Pigs

Swine are among the most sensitive species to mycotoxins. The swine immune response to AF has been inconsistent. The swine humoral immune response was not altered by feeding mixed AF at levels ranging from 0.4 to 0.8 mg/kg of feed to acutely toxic levels as high as 500 mg/kg of feed. The immunosuppression caused by AF (140 or 280 μg/kg of feed) only occurs at the cellular and not the humoral level and the inhibition of DNA synthesis in porcine lymphocytes when AFB_1 was added to the medium at various levels (0.1–10 000 ng/ml of medium) (Pang and Pan, 1994). Negative effects of the mycotoxin ZEN on swine reproductive function have been demonstrated (Diekman and Green, 1992). Pigs have been shown to draw the toxic forms of ZEN back from the circulating glucuronide conjugate. For this reason the oestrogenic effects of ZEN have been pronounced and prolonged in pigs. An extensive study in Hungarian farms showed swelling of the vulva and mammary glands and occasional vaginal and rectal prolapses in sexually mature gilts consuming feed contaminated with ZEN (Glavitis and Vanyi, 1995). Other oestrogenic effects of ZEN on gilts or sows included edematous uterus, ovarian cysts, increased follicular maturation and number of stillborns, and decreased fertilization rate. In the same study, ZEN induced germinal epithelial degeneration and altered sperm formation in boars. Reproductive disorders (e.g. atrophy of the ovaries and uterus, ovarian degeneration, and glandular dysfunction of the endometrium) also have been reported when sows were exposed to feed contaminated with T-2 toxin. Signs of prenatal T-2 toxicosis (e.g. glandular dysfunction of the endometrium, gastrointestinal edema, and hematopoesis leading to death) were also observed in suckling piglets (Hussein and Brasel, 2001).

4.3. Horses

The history of myctoxicosis and poisoning in equine has been reviewed by Asquith (1991). In a case study, mature horses

consuming AFB_1-contaminated feed (58.4 μg/kg) were jaundiced and anorexic before death. Post-mortem examinations revealed enlarged livers, kidney damage, and lesions of bile-duct hyperplasia. In other cases, equine aflatoxicosis has been characterized by depression, lameness, and death. Post-mortem examinations revealed subcutaneous and enteric hemorrhage, enlarged kidneys, enlarged necrotic livers, and hepatic, nephritic, and myocardial lesions. Studies with ponies have shown damage in the skeletal muscles and heart along with liver dysfunction when acute lethal doses of AFB_1 were administered. Post-mortem examination of horses consuming corn contaminated with a mixture of AF (AFB_1, AFB_2, and AFM_1 at 114, 10, and 6 μg/kg, respectively) revealed severe hepatic lesions (Vesonder et al., 1991).

The greatest mycotoxin risks to equine identified thus far are the toxins produced by *Fusarium moniliforme* which has been implicated in equine leukoencephalomalacia and acute neurotoxicity. These diseases were attributed to consumption of corn contaminated with FB_1 and moniliformin toxins. Symptoms of equine leukoencephalomalacia include ataxia, paresis, apathy hypersensitivity, impaired locomotor function, necrosis of cerebral white matter, and lesions in the cerebral cortex. Bean-hulls poisoning is another mycotoxin-related disease that has been known in Hokkaido (Japan) for seven decades because of the availability of bean-hulls as a cheap source of feed and bedding for horses (Asquith, 1991). Clinical symptoms include central nervous system dysfunction, rapid heartbeat, diminished ocular reflexes, and death (Placinta et al., 1999).

4.4. Dogs and cats

The effects of mycotoxins on companion animals are severe and can lead to death. As early as 1952, a case of hepatitis in dogs was directly linked to consumption of moldy food. Following the discovery of AF, the agent responsible for the 1952 case was identified as AFB_1 and the symptoms of aflatoxicoses in dogs were elucidated. In the case study, three dogs on a farm in Queensland became ill (severe depression, anorexia, and weakness) and died at different times within a month following consumption of a commercial dog food mixed with AF-contaminated bread. The vomitus specimens from one dog contained high levels of AF (100 μg/g of AFB_1 and 40 μg/g of AFG_1) (Devegowda and Castaldo, 2000). Deoxynivalenol is a major health concern for companion animals and it contaminates petfood via corn even after processing. Due to the variable toxicity responses to DON in dogs and cats, it was suggested that DON levels in petfood should not exceed 0.5 μg/kg. In a case study, T-2 toxin given to cats intravenously at 2 mg/kg resulted in hypovolemia and death. Sub-lethal T-2 toxicity in cats has been shown to lower white blood cell counts (Devegowda and Castaldo, 2000).

As with other species, the kidney is the primary target organ of OTA in dogs and cats. In a study with dogs, pacing and vomiting were observed at an OTA dose of 0.2 mg/kg. At doses between 0.2 and 3.0 mg/kg symptoms of intoxication in dogs included anorexia, polydipsea, polyuria, anxiety, prostration, and death. The necropsy findings included epithelial degeneration (proximal tubules), mucohemorrhagic enteritis (cecum, colon, and rectum) and necrosis of the lymphoid tissues (spleen, tonsil, thymus, and peripheral lymph nodes) (Bird, 2000).

4.5. Rats and mice

Rats have been used extensively for decades as a model for human mycotoxicoses especially with regard to the carcinogenic potential of AF. This model system, however, has been a subject for debate due to the differences in the detoxification mechanisms between rats and humans as shown by cytosolic conjugation of AFB_1 in vitro (Raney et al., 1992). In contrast to rats, mice are generally resistant to the hepatocarcinogenic effects of AFB_1. This may explain the high level of glutathione-*S*-transferase (GST) activity in mice challenged with AFB_1 (Quinn et al., 1990). Contrary to the hepatocellular carcinomas commonly found in rat studies with AFB_1, mice given AFB_1 by intraperitoneal injection at 0.02 mg/kg of body weight for 12 injections over 3 weeks (average 5.6 mg/kg body weight) have expressed pulmonary tumors. Other mycotoxins such as FB_1 also have been implicated in hepatic tumor formation in rats (Gelderblom and Snyman, 1991).

The negative effects of trichothecenes on rats have been known for decades and, as a result, the rat has been used extensively as a model for trichothecene toxicity tests. Studies with T-2 toxin have shown the LD_{50} of its oral administration to range from 2.8 to 3.8 mg/kg (Kravchenko et al., 1983). Oral administration of T-2 toxin at levels ranging from 5 to 25 μg/kg of feed for extended periods (up to 16 week) reduced feed intake in a dose-dependent manner and caused gastric ulcers, thymic depression and reduced nutrient uptake and lipid metabolism (i.e. elevated levels of triglycerides, free cholesterol, total phospholipids, and phosphatidyl choline (Suneja et al., 1984). Symptoms of acute T-2 toxicity in rats include lethargy, reduced feed intake, decreased body temperatures, increased number of white blood cells and lymphocytes by threefold, hypertension, and finally tachycardia precedes hypotension and death (Wannemacher et al., 1991).

5. Negative effects of mycotoxins on ruminants

Ruminants such as cattle, sheep, goats, and deer are less known for their sensitivity to the negative effects of mycotoxins than are non-ruminants. However, production (milk, beef, or wool), reproduction, and growth can be altered when ruminants consume mycotoxin-contaminated feed for extended periods of time (Hussein and Brasel, 2001).

5.1. Cattle

Aflatoxins have been shown to negatively effect production, immune system function, and rumen metabolism in cattle. Increasing AF in cattle feed to levels such as 10, 26, 56.4, 81.1, and 108.5 μg/kg has been shown to significantly reduce feed intake at each level in a dose-dependent manner (Choudhary et al., 1998). In a 155-day feeding trial, AFB_1 (600 μg/kg) was shown to depress feed efficiency and rate of gain in steers. Decreased feed efficiency in cattle has been attributed to compromised ruminal function by reducing cellulose digestion, volatile fatty acid (i.e. acetate, propionate, and butyrate) production and rumen motility (Diekman and Green, 1992).

Several mechanisms of bovine immunosuppression by AFB_1 have been illustrated in vitro; Paul et al. (1977) demonstrated

that AFB_1 suppressed mitogen-induced stimulation of peripheral lymphocytes. In another study (Bodine et al., 1984), AFB_1 was shown to inhibit bovine lymphocyte blastogenesis. In a study by Cook et al. (1986), radiotelemetry was used to measure rumen motility in cattle and the results showed that AF administration (200–800 μg/kg) slowed rumen motility in a dose-dependent manner (Cook et al., 1986). Ochratoxins, on the other hand, do not cause significant toxicity to cattle when fed alone in naturally occurring doses. Barley naturally-contaminated with OTA (390–540 μg/kg) and low levels of AFB_1 (12–13 μg/kg) did not induce any significant clinical symptoms in 12-week-old calves. The absence of a toxic effect may have been due to the ruminal microbial degradation and detoxification (Patterson et al., 1981).

Aflatoxins also affect the quality of milk produced by dairy cows and result in carry-over of AFM_1 from AF-contaminated feed. Ten ruminally-canulated lactating Holstein cows were given AFB_1 (13 mg per cow daily) via the rumen orifice for 7 days. Levels of AFM_1 in the milk of the treated cows ranged from 1.05 to 10.58 ng/l. The AFB_1-treated cows also had a significant reduction in milk yield. The carry-over rate was shown to be higher (6.2 vs. 1.8) in early lactation (2–4 weeks) when compared with late lactation (34–36 weeks) (Veldman et al., 1992). The T-2 toxin is also believed to induce immunosuppression in cattle by decreasing serum concentrations of IgM, IgG, and IgA, neutrophil functions and lymphocyte blastogenesis, and the response of lymphocytes to phytohemagglutinin (Mann et al., 1984). This toxin was also shown to induce necrosis of lymphoid tissues. Bovine infertility and abortion in the final trimester of gestation also have resulted from consumption of feed contaminated with T-2 toxin (Placinta et al., 1999). Calves consuming T-2 toxin at 10–50 mg/kg of feed have demonstrated ulcers in the abomesum and sloughing of the papilla in the rumen (Cheeke, 1998a).

A case investigation of dairy cattle fed moldy corn containing 1 mg/kg T-2 toxin resulted in hemmorhagic syndrome. With the exception of T-2 toxin, cattle have not been adversely affected by trichothecenes. Neither DON nor DAS are known to affect cattle health or performance in the feedlot (Dicostanzo et al., 1996). It has shown that DON at levels as high as 6 mg/kg of feed had no adverse effects on milk yield and did not show evidence of carry-over into milk. Zearalenone has been suggested as a causative agent of infertility, reduced milk production, and hyperestrogenism in cattle (D'Mello and MacDonald, 1997).

Fescue foot, hyperthermia, and fat necrosis in cattle have been linked to consumption of tall fescue parasitized with *Acremonium coenophialum* (Cheeke, 1998b). Fescue foot in cattle has been shown to derive from vasoconstriction and gangrene in the hooves and tail due to the relaxation of smooth muscles caused by ergot alkaloids. Hyperthermia (summer fescue toxicosis) in cattle has been characterized by symptoms of weight loss, salivation, and heat stress. Fat necrosis in cattle is a condition in which areas of the fat are hardened resulting in constriction of internal organs, reduced serum cholesterol, and elevated serum amylase (Cheeke, 1998b). Cattle consuming tall fescue contaminated with endophytic fungi such as *Acremonium lolii* also have shown symptoms of staggers, excitability, increased rectal temperature, increased respiration rate, and loss of body weight (Ross et al., 1989).

5.2. Sheep

Early studies suggested that the sheep are the most resistant species to mycotoxicosis (Miller and Wilson, 1994). However, feeding diets contaminated with AF (79% AFB_1, 16% AFG_1, 4% AFB_2, and 1% AFG_2) to ewe lambs (2.5 mg/kg or 5.0 mg/kg of feed for 35 days) resulted in hepatotoxicity (Harvey et al., 1995). In another study (Fernandez et al., 1997), lambs fed AF at 2.5 mg/kg of feed daily for 21 days showed symptoms of clinical aflatoxicosis including hepatic and nephritic lesions, altered mineral metabolism, and increased size and weight of the liver and kidney. Another study (Ramos et al., 1996) with the same daily dose of AF (2.5 mg/kg of feed) examined the plasma mineral concentrations on day 1, 2, 4 and 8 of the initial dose. On day 4 of intoxication, significant reductions in plasma mineral concentrations were detected for Ca (2.39 vs. 2.06 mM), P (2.95 vs. 2.50 mM), Mg (0.88 vs. 0.77 mM), K (4.40 vs. 3.81 mM), and Zn (13.2 vs. 11.6 μM). The resulting mineral deficiencies due to aflatoxicosis were attributed to lower feed intake and to the liver and kidney malfunctions as a result of AF intoxication. Exposure of lambs to AF (2.5 mg/kg of feed for 3 weeks) revealed changes in extrinsic coagulation factors as determined by increased fibrinogen concentration (Fernandez et al., 1997).

Mechanisms for cellular immune response to AF in sheep have not been elucidated. *Fusaria* mycotoxins at high doses also appear to have some negative effects on sheep. Exposing sheep to DON (15.6 mg/kg of feed) for 28 days had no effects on average daily gain, hemacytology parameters, or liver function. However, weight loss (−0.6 vs. 2.4 kg/day) was reported after 34 days of feeding DAS (5 mg/kg of feed) to lambs. Further weight loss (−2.7 vs. 2.4 kg/day) also was reported at 34 days of feeding lambs same level of DAS in combination with AF (2.5 mg/kg of feed) suggesting a synergistic effect (Harvey et al., 1995). It has been suggested that high dietary levels (12 mg/kg of feed) of ZEN for extended periods of time (10 days) may affect reproductive performance of sheep negatively by reducing fertility and ovulation rates (Dicostanzo et al., 1996). Fumonisins at high doses (11.1–45.5 mg/kg of body weight) have been demonstrated as acutely and fatally nephrotoxic and hepatotoxic in lambs (Edrington et al., 1995). It should be noted, however, that such experimental levels have not been found in F-contaminated feeds.

Sheep also have been affected by ryegrass toxicosis, which has resulted in tremors, decreased productivity, and in some cases death (D'Mello and MacDonald, 1997). Perennial ryegrass staggers have been observed in sheep consuming ryegrass contaminated with *A. lollii*. Symptoms have included shaking with loss of coordination and inability to walk (Cheeke, 1998b). Staggers have been demonstrated when *A. lolii*-contaminated ryegrass had lolitrem B toxin at levels of 2.0–2.5 mg/kg (DiMenna et al., 1992).

5.3. Other ruminants

Ruminants other than cattle and sheep have shown variable resistance to mycotoxins. Levels of AF at 95 mg/kg of feed offered to weanling goats had no effects on body weight gain and did not show any noticeable signs of toxic effects (Gurung et al., 1998). Signs of toxic effects were only detected through serum profile and sphingolipid analysis. In a study with white-tailed

deer fawn fed 800 mg/kg AF over an 8-week-period (Quist et al., 1997), acute injuries in the liver were indicated by increased serum bile acid concentrations and hepatic lesions.

6. Factors affecting production, contamination of foods and feeds, and toxicity of mycotoxins

A main difficulty in assessing the risk of mycotoxins to human and animal health is the multiplicity of factors affecting the production or presence of mycotoxins in foods or feeds. Mere isolation and confirmation of mycotoxigenic fungal species in foods or feeds does not indicate the presence of mycotoxins. Upon development of accurate and sensitive techniques for qualitative and quantitative analysis of mycotoxins, researchers have found that various factors operate interdependently to affect fungal colonization and/or production of the mycotoxins. D'Mello and MacDonald (1997) categorized the factors as physical, chemical, and biological.

Physical factors include the environmental conditions conducive to fungal colonization and mycotoxin production such as temperature, relative humidity, and insect infestation. Chemical factors include the use of fungicides and/or fertilizers. Stresses such as drought, an increase in temperature, and an increase in relative humidity may selectively alter colonization and metabolism of mycotoxigenic fungi and thus alter mycotoxin production (Russell et al., 1991). These researchers also indicated that unseasonable conditions may render crops and forages susceptible to mycotoxin production. Cool and damp springtime weather favours the germination of the sclerotia and thus ergot alkaloid formation in fescue and ryegrass (Cheeke, 1998a).

The biological factors are based on the interactions between the colonizing toxigenic fungal species and substrate. While some plant species are more susceptible to colonization, environmental conditions may increase the vulnerability of other more resistant plant species. The biological factors have been further sub-categorized (Moss, 1991) into intrinsic factors including fungal species, strain specificity, strain variation, and instability of toxigenic properties. Such intrinsic factors underscore the difficulty of risk assessment of mycotoxin exposure based on mold contamination. Species and strain specificity are well described by the numerous mycotoxins produced by two or more fungi. A strain variation refers to a specific culture identity for the same species fungal isolate and how these strains produce mycotoxins in a variable fashion. Finally, the toxigenic properties may vary over time and as the mycoecology changes toxins may be reduced. Several studies have shown that optimal conditions for fungal growth are not necessarily optimum for toxin production. For example, different strains of *A. flavus* have been shown to produce AF at different rates when cultured under similar conditions (Hesseltine et al., 1970).

In a 3-year study (Wood, 1992) on occurrence of AF in selected USA foods (milled corn products, peanuts, and peanut products) and feeds (shelled corn, cottonseed, and cottonseed meal), the unpredictable nature of AF contamination and the difficulty in assessing the extent of such contamination have been documented. Very high levels (greater than the 20 μg/kg) of contamination were found in corn harvested from all parts of the USA in 1989, 1990, and 1991. In these years, 9.1%, 20.7%, and 37.1%, respectively, of the corn samples examined before human consumption contained AF at levels >20 μg/kg in the southern states (i.e. Arkansas, Texas, and Oklahoma). The increase in AF contamination in 1990 was attributed to the drought condition. However, the dramatic increase in AF contamination in 1991 could not be explained by environmental factors. In an earlier study by Wood (1989), it was found that cottonseed and cottonseed meal were the primary targets of AF contamination in the southwestern states of Arizona and California. In comparison to the rest of the nation (0 and 28.0 μg/kg for cottonseed and cottonseed meal, respectively), the average values for both states were 37.7 and 43.0 μg/kg of seed and meal, respectively. In a more practical sense, mycotoxin contamination of foods or feeds may result from inadequate storage and/or handling of harvested products. Prevention and control methods have been prescribed for mitigating mycotoxin contamination of feeds (Harris, 1997). These methods require that feed handlers and grain mill operators keep grain bins clean and store grain at less than 14% moisture. Feed ingredients must be dry, oxygen free, fermented or treated with mold growth inhibitors. With regard to silage crops, harvesting at the appropriate moisture content and both packing and sealing the silo (to exclude oxygen and allow for desirable anaerobic fermentation) are essential for reducing mycotoxin contamination potential.

Chemical treatment and processing are anthropogenic factors that may decrease mycotoxin contamination of foods or feeds. Wet and dry milling processes as well as heat in the cooking process have been shown to reduce AF in foods. Heating and roasting have been shown to significantly decrease AF content in corn. A review of several studies, however, suggested that processing and pasteurization of milk do not completely destroy mycotoxins (Manorama and Singh, 1995). Bentonite and aluminosilicate clays used as binding agents have been shown to reduce AF intoxication in pigs, cattle, rats, and poultry without causing digestive problems when mixed with AF-contaminated feeds (Scheideler, 1993). However, these clays are ineffective against ZEN and F, can alter the nutritional value (by binding trace minerals and vitamins and reducing their bioavailability), and produce dioxins (Devegowda and Castaldo, 2000).

Recently, the potential role of dietary factors to counteract the toxic effects of mycotoxins has been reviewed (Galvano et al., 2001). The role of antioxidants (Se and vitamins A, C, and E) and food additives was evaluated. Antioxidant defense mechanisms observed have included free radical scavenging, reduced lipid peroxidation, and general inhibition of the mutagenic process. Galvano et al. (2001) also reviewed the role of food components (fructose, phenolic compounds, coumarins, and chlorophyll) and food additives (piperine, aspartame, cyproheptadine, and allyl sulfides) in reducing the toxicity of various mycotoxins by decreasing toxin formation and enhancing metabolism. For example, phenolic compounds have been shown to metabolically enhance AFB_1 conjugation and elimination (Rompelberg et al., 1996).

The antioxidant ethoxyquin has been recognized as a strong anti-aflatoxigenic agent. Kensler et al. (1986) demonstrated the role of ethoxyquin in rat hepatocytes as induction of conjugating GST. Mendel et al. (1987) confirmed the enhancing effect of ethoxyquin on phase II metabolism in several subcellular components (microsomes, cytosol, and cell membrane) of the rat liver. In another study, gamma glutamyl transpeptidase was induced along with GST. A more recent study with marmosets has established ethoxyquin as a potential chemoprotective

agent against the carcinogenic effects of AFB_1 in humans (Bammler et al., 2000).

7. Economic impact of mycotoxins

There are multiple criteria for assessing the economic impact of mycotoxins on humans and on animals. Considerations include loss of human and animal life, health care and veterinary care costs, loss of livestock production, loss of forage crops and feeds, regulatory costs, and research cost focusing on relieving the impact and severity of the mycotoxin problem. Formulas for worldwide economic impact have been difficult to develop and, therefore, most reports on economic impact are on a single aspect of mycotoxin exposure or contamination (Hussein and Brasel, 2001). The worldwide contamination of foods and feeds with mycotoxins is a significant problem. Studies have shown extensive mycotoxin contamination in both developing and developed countries. Surveillance studies (Placinta et al., 1999) showed that worldwide contamination of cereal grains and other feeds with *Fusarium* mycotoxins is a global concern. In Yugoslavia, studies on mycotoxigenic fungi in raw milk have indicated that 91% of the samples tested were contaminated (Skrinjar et al., 1995). In the USA, a study was conducted in seven Midwestern states in 1988–1989 and found mycotoxins in 19.5% of corn samples assayed prior to any induced environmental stress and 24.7% of the samples following stress induction (Russell et al., 1991). Shane (1994) estimated the 1980 losses due to AF in corn of eight Southeastern states at 97 million dollars with additional 100 million dollars in production losses at hog farms feeding the contaminated corn.

India is a prime example of a country in which the economy is affected heavily by mycotoxins. In a study in the Bihar region from 1985 to 1987 (Ranjan and Sinha, 1991), nearly 51% of the 387 samples tested were contaminated with molds. Of the 139 samples containing AF, 133 had levels above 20 μg/kg. In another study (Phillips et al., 1996), levels as high as 3700 μg/kg of AF was reported in groundnut meal used for dairy cattle. Researchers also found 21 of 28 dairy feed samples from farms in and around Ludhiana and Punjab to be contaminated with AFB_1 at levels ranging from 50 to 400 μg/kg (Dhand et al., 1998). It was estimated that 10 million dollars were lost in India's export within a decade due to groundnut contamination with mycotoxins (Vasanthi and Bhat, 1998).

8. Regulation of mycotoxins in foods and feeds

Other than the direct health risk, economic losses and implications arising from mycotoxicoses are enormous. Many developing countries have realised that reducing mycotoxins levels in foods will not only reduce financial burden on health care but also confer international trade advantages such as exports to the attractive European markets. Factors fundamental to country's ability to protect its population from mycotoxins include the political will to address mycotoxins exposure and capability to test food for contamination, which determines whether requirements can be enforced (Wagacha and Muthomi, 2008).

8.1. Good agricultural practices

Agronomic practices have been shown to have profound effect on mycotoxins contamination of crops in the field.

(i) *Early harvesting: early* harvesting reduces fungal infection of crops in the field before harvest and consequent contamination of harvested produce. Even though majority of farmers in Africa are well aware of the need for early harvesting, unpredictable weather, labour constraint, need for cash, threat of thieves, rodents and other animals compel farmers to harvest at inappropriate time (Amyot, 1983). Rachaputi et al. (2002) reported that early harvesting and threshing of groundnuts resulted in lower aflatoxin levels and higher gross returns of 27% than in delayed harvesting.

(ii) *Proper drying: rapid* drying of agricultural products to low moisture level is critical as it creates less favourable conditions for fungal growth and proliferation, insect infestation and helps keep longer (Lanyasunya et al., 2005). Hamiton (2000) reported that drying harvested maize to 15.5% moisture content or lower within 24–48 h would reduce the risk of fungal growth and consequent aflatoxin production. Awuah and Ellis (2002) demonstrated that when groundnuts were dried to 6.6% moisture level, they were free of fungi regardless of the local storage protectant used for 6 months, whereas at 12% moisture, only jute bags with the plant *Syzigum aromaticum* effectively suppressed the cross infection of healthy kernels. However, when the moisture content was increased to 18.5%, the latter treatment was not as effective. A community-based intervention trial in Guinea, West Africa focused on thorough drying and proper storage of groundnuts in subsistence farm villages and achieved a 60% reduction in mean aflatoxin levels in intervention villages (Turner et al., 2005). During storage, transportation and marketing, maintenance of low moisture levels should be maintained by avoiding leaking roofs and condensation arising from inadequate ventilation.

(iii) *Physical treatment: a* study conducted in Benin by Fandohan et al. (2005) to determine the fate of aflatoxins and fumonisins through traditional processing of naturally-contaminated maize and maize based foods, demonstrated that sorting, winnowing, washing, crushing combined with de-hulling of maize grains were effective in achieving significant mycotoxins removal. Similar results have been reported by Park (2002) and Lopez-Garcia and Park (1998). This approach is based on separation of contaminated grain from the bulk and depends on the heavy contamination of only a small fraction of the seeds, so that removing those leaves a much lower overall contamination. Study of the distribution of aflatoxin in peanuts shows that a major portion (80%) of the toxin is often associated with the small and shrivelled seed and moldy and stained peanut (Fandohan et al., 2005; Turner et al., 2005).

(iv) *Sanitation*: basic sanitation measures such as removal and destruction of debris from previous harvest would help in minimizing infection and infestation of produce in the field. Cleaning stores before loading new produce has been shown to be correlated with reduced aflatoxin levels (Hell et al., 2000).

(v) *Proper storage: to* preserve quality in storage, it is necessary to prevent biological activity through adequate drying to less than 10% moisture, elimination of insect activity that can increase moisture content through

condensation of moisture resulting from respiration, low temperatures, and inert atmospheres (Lanyasunya et al., 2005; Turner et al., 2005).

(vi) *Insect management: the* level of insect damage influences the extent of mycotoxins contamination. Avantaggio et al. (2002) found that insect damage of maize is good predictor of *Fusarium* mycotoxins contamination. Insects carry spores of mycotoxins producing fungi from plant surfaces to the interior of the stalk or kernels or create infection wounds through their feeding habits (Munkvold, 2003). Therefore, proper management of insect pests through any appropriate control strategy would reduce mycotoxins contamination problem.

(vii) *Othermethods: cultural* practices including crop rotation, tillage, planting date, and management of irrigation and fertilization, have limited effects on infection and subsequent mycotoxins accumulation (Munkvold, 2003; Champeil et al., 2004).

8.2. Biological control

Significant inroads have been made in establishing various bio-control strategies such as development of atoxigenic bio-control fungi that can out-compete their closely related, toxigenic strains in field environments, thus reducing the levels of mycotoxins in the crops (Cleveland et al., 2003). Dorner and Cole (2002) reported a field application of non-toxigenic strains of *A. flavus* and *A. parasiticus* that reduced post-harvest aflatoxin contamination by 95.9%. Use of biological agents to suppress growth of fumonisin-producing fungi has been reported. It was observed that the inhibition of fumonisin formation by atoxigenic *F. verticillioides* strains although these caused higher disease incidence when applied through the silk channel. The observation implied that the ability to produce fumonisins is not required to produce ear rot and that effective colonization of plant with atoxigenic strains could competitively exclude fumonisin-producing strains or prevent them from producing fumonisins. Luongo et al. (2005) also reported suppression of saprophytic colonization and sporulation of toxigenic *F. verticillioides* and *F. proliferatum* in maize residues by non-pathogenic *Fusarium* species. Control of fumonisin-producing fungi by endophytic bacteria has also been reported.

Competitive exclusion, whereby the bacteria grow intercellularly precluding or reducing growth of intercellular hyphae was thought to be the mechanism involved. Masoud and Kaltoft (2006) reported in vitro inhibition of OTA production by *A. ochraceus* by three yeasts (*Pichia anomala*, *Pichia kluyveri* and *Hanseniaspora uvarum*). Fungal strains of *Trichoderma* have also been demonstrated to control pathogenic fungi through mechanisms such as competition for nutrients and space, fungistasis, antibiosis, rhizosphere modification, mycoparasitism, biofertilization and the stimulation of plant-defense mechanisms (Benitez et al., 2004). The ability of fungal antagonists to control toxigenic types is, however, dependent on the differential effect of macro and micro-climatic conditions on the antagonist–pathogen interaction (Luongo et al., 2005).

Important criteria for evaluating the effectiveness of mycotoxin bio-control agent include ability to colonize the target substrate or plant part, ability to be active under various environmental conditions in the field or during storage so that its growth and that of the pathogen coincide and compatibility with other control procedures without inducing effects that compromise end use quality of the commodity (Bacon et al., 2001). In this regard, atoxigenic strains of *F. verticillioides* and *F. proliferatum* would be superior bio-control agents for toxigenic strains since they occupy the same ecological niche as the toxigenic strains in the host plant and share similar growth conditions.

8.3. Chemical control

Appropriate use of pesticides during the production process could help in minimizing the fungal infection or insect infestation of crops and consequently mycotoxin contamination. Fumonisins contamination could be reduced by application of fungicides that have been used in control of Fusarium head blight such as prochloraz, propiconazole, epoxyconazole, tebuconazole cyproconazole and azoxystrobin (Matthies and Buchenauer, 2000; Haidukowski et al., 2004). On the other hand, fungicides such as itraconazole and amphotericin B have been shown to effectively control the aflatoxin-producing *Aspergillus* species (Ni and Streett, 2005). However, use of fungicides is being discouraged due to economic reasons and growing concern for environment and food safety issues.

8.4. Decontamination

Decontamination of food/feed contaminated with mycotoxins could be achieved through either chemoprotection or enterosorption. Chemoprotection of aflatoxins has been demonstrated with the use of a number of chemical compounds like oltipraz and chlorophylin or dietary intervention like broccoli sprouts and green tea that either increase an animal's detoxification processes (Kensler et al., 2004) or prevent the production of the epoxide that leads to chromosomal damage. This intervention might not however be sustainable in the long-term in most African countries since it involves drug therapies, which are expensive besides the possible side effects. Entersorption is based on the discovery of certain clay minerals, such as Novasil, which can selectively adsorb mycotoxins tightly enough to prevent their absorption from the gastrointestinal tract (Wang et al., 2005).

There are different adsorption agents but their efficacy in preventing mycotoxicosis varies. Selected calcium montorillonites have proven to be the most highly selective and effective of enterosorbents. However, with enterosorption, there is a risk that non-specific adsorption agents may prevent uptake of micronutrients from the food. Essential oils and aqueous extracts of *Aframomum danielli* were recently reported to reduce OTA in spiked cocoa powder by between 64% and 95%. Although ochratoxin molecule is stable, it is acknowledged that around 40–90% of OTA is destroyed during roasting of coffee beans (Aroyeun and Adegoke, 2007).

8.5. Breeding for resistance

This is one of the most promising long-term strategies in mycotoxins contamination menace in Africa. Sources of resistance to *A. flavus* and *Fusarium* spp., particularly *F. verticillioides* have been identified and have been incorporated into public and private breeding programs (Munkvold, 2003). Potential biochemical and genetic resistance markers have been identified in crops, particularly maize in different parts of the world which are being utilized as selectable markers in breeding for

resistance to aflatoxin contamination. Prototypes of genetically engineered crops have been developed which (a) contain genes for resistance to the phytotoxic effects of certain trichothecenes, thereby helping reduce fungal virulence or (b) contain genes encoding fungal growth inhibitors for reducing fungal infection in the USA. Gene clusters housing the genes that govern formation of trichothecenes, fumonisins and aflatoxins have been elucidated and are being targeted in strategies to interrupt the biosynthesis of these mycotoxins (Cleveland et al., 2003) Scientists at United States Department of Agriculture have identified two maize lines that are resistant to *A. flavus* and *F. moniliforme* (Hamiton, 2000).

However, few if any, commercial cultivars have adequate levels of resistance to mycotoxins producing fungi (Munkvold, 2003). Many organizations such as IITA are continuously working on resistance breeding programs in Africa (Hell et al., 2005). To devise effective strategies to control fungal infection and minimize mycotoxin production in host plants, a better knowledge of genetic variability and population structure at the intra-specific level and ability to detect cryptic populations or lineages which might arise that possess significant features in terms of toxin profile or host preferences is necessary (Mule et al., 2005).

8.6. Legislation

Mycotoxin regulations have been established in about 100 countries, out of which 15 are African, to protect the consumer from the harmful effects of these mycotoxins (Van Egmond, 2002; Barug et al., 2003; Fellinger, 2006). Human foods are allowed 4–30 ppb aflatoxin, depending on the country involved (FDA, 2004). In the USA, 20 μg/kg is the maximum aflatoxin residue limit allowed in food for human consumption, except for milk (Wu, 2006) while 4 μg/kg total aflatoxin in food for human consumption are the maximum acceptable limits in the EU, the strictest in standard worldwide (EC, 2006; Wu, 2006). OTA has been evaluated at the 37th, 44th and 56th meetings of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and a provisional tolerable weekly intake (PTWI) of 100 ng/kg body weight has been established (Benford et al., 2001). The European Union (EU) has recently issued a proposal to lower maximum tolerated limits for several mycotoxins in food and feed which became effective from 1st October 2006 (EC, 2006).

8.7. Surveillance and awareness creation

This could be a long-term intervention strategy as has been advocated by WHO (2006) and James (2005). It is imperative for African countries to strengthen nationwide surveillance, increase food and feed inspections to ensure food safety, and local education and assistance to ensure that food grains and animal feeds are harvested correctly, dried completely, and stored properly. Awareness of what mycotoxins are and the dangers that they pose to human and animal health could be done through government bodies, private organizations, non-governmental organizations, national media networks such as radios and television programs as well as features in newspapers and magazines. Seminars and workshops could be used as avenues and bridges of information exchange and dissemination between researchers and the populace respectively. Such events also serve as forums to assess past and present work and define and streamline areas of future studies. WHO (2006), has put plans in place to focus on field projects, strengthening surveillance and awareness raising and educating consumers on matters related to mycotoxins in Africa among others. It is imperative that critical evaluation of the intervention strategies is done to put into consideration the sustainability, cultural acceptability, economic feasibility, ethical implication, and overall effectiveness of potential interventions.

Considering the challenges and the current needs, a regional experts meeting in 2005 on aflatoxins problem with particular reference to Africa made certain recommendations that could be instrumental in addressing or reducing mycotoxins contamination in the continent. The consultation noted that the achievement of mycotoxins reduction and control is dependent on the concerted efforts of all actors along the food production chain. Multidisciplinary approaches are therefore critical. The meeting recommended continued mycotoxins awareness as a public health issue, strengthened laboratory and surveillance capacities as well as establishing early warning systems. The participants also identified several research needs including cost-benefit analysis of interventions and research on the occurrence of mycotoxins in foods. In addition, there remains a need for efficient, cost-effective sampling and analytical methods that can be used for the detection of mycotoxins in developing countries (WHO, 2006).

9. Conclusion

Fungi cause human illness in different ways. Mycoses are the best-known diseases of fungal etiology, but toxic secondary metabolites produced by saprophytic species are also an important health hazard. The term mycotoxin is an artificial rubric used to describe pharmacologically active mold metabolites characterized by vertebrate toxicity. They fall into several chemically unrelated classes, are produced in a strain-specific way, and elicit some complicated and overlapping toxigenic activities in sensitive species that include carcinogenicity, inhibition of protein synthesis, immunosuppression, dermal irritation, and other metabolic perturbations. Mycotoxins usually enter the body via ingestion of contaminated foods, but inhalation of toxigenic spores and direct dermal contact are also important routes.

It is difficult to prove that a disease is a mycotoxicosis. Molds may be present without producing any toxin. Thus, the demonstration of mold contamination is not the same thing as the demonstration of mycotoxin contamination. Moreover, even when mycotoxins are detected, it is not easy to show that they are the etiological agents in a given veterinary or human health problem. Nevertheless, there is sufficient evidence from animal models and human epidemiological data to conclude that mycotoxins pose an important danger to human and animal health, albeit one that is hard to pin down. The incidence of mycotoxicoses may be more common than suspected. It is easy to attribute the symptoms of acute mycotoxin poisoning to other causes; the opposite is true of etiology. It is not easy to prove that cancer and other chronic conditions are caused by mycotoxin exposure. In summary, in the absence of appropriate investigative criteria and reliable laboratory tests, the mycotoxicoses will remain diagnostically daunting diseases.

Adaptation in higher trophic levels, such as mammalian species, has been crucial to survival as well. In view of the wide

range and varying degrees of mycotoxicoses in animals, there have been signs of co-evolutionary aspects of mammalian susceptibilities. Monogastric animals from fowl to domestic companion animals to humans have been susceptible to a number of mycotoxins. Besides the loss in productivity and toxicity tests with very high doses, studies demonstrated a relative resistance of ruminants to the adverse effects of a number of mycotoxins, opening the door for research into adaptive mechanisms in these species.

The key to determining how the ruminant species adapted to mycotoxins has been through studies on the metabolic pathways in the rumen environment. Besides the demonstrated effects of mycotoxins on humans or animals some important aspects of toxicology and control have still resided in the realm of the unknown and unexplored. For example, there has been a general paucity of data on mycotoxins classified as carcinogens in humans by IARC, and currently there is a genuine concern over the carcinogenic potential of OTA and F, for which few regulations exist worldwide. Only with continued research on understanding the effects and modes of mycotoxin action in various species, have regulations and control strategies been forthcoming.

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