Ass. Univ. Bull. Environ. Res. Vol. 7 No. 1, March 2004

AUCES

MYCOTOXINS IN FOODS AND FEEDS
1-AFLATOXINS

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REVIEW ARTICLE

ABSTRACT :

Mycotoxins are toxic metabolites synthesized by some naturally occurring fungi under suitable physical, chemical and biological factors. High temperature stress, humidity stress and insect damage of the product are major determining factors in mold infestation and toxin production. Mycotoxins contaminated food and feed supplies could increase the economic and health risks to humans and animals. The aflatoxins constitute a group of fungal metabolites that have varied toxic and carcinogenic properties, depending on dose and duration of exposure.

The adverse effects of aflatoxins in humans ranged from acute hepatic toxicity to chronic disease such as liver cancer. In animals, the aflatoxins cause liver damage, decreased milk production, reduced reproductively and suppressed immunity in animals consuming low dietary concentrations. In acute toxicity the clinical signs include gastrointestinal dysfunctions, decreased feed intake and efficiency, weight loss, jaundice, drop in milk production, nervous signs, bleeding and death. All species of animals are susceptible to aflatoxicosis. The susceptibility of individual animals to aflatoxicosis varies considerably depending on dose, duration of exposure, species, age, sex and nutrition.

In poultry, beside inappetance, weight loss, decreased egg production, leg and bone problems, poor pigmentation, fatty liver, kidney dysfunction, bruising and death, suppression to natural immunity and susceptibility to parasitic, bacterial and viral infections can occur.

INTRODUCTION:

Aflatoxins B_1, B_2, G_1 and G_2 are produced by three molds of the Aspergillus species: *A. flavus* (A+fla+toxin), *A. parasiticus* and *A. nomius* and various species of Penicillium, Rhizopus, Mucor and Streptomyces, which contaminate plants and plant products (Smith, 2002; WHO, 1998). *Aspergillus flavus* and *A. parasiticus* are common in most soils and are usually involved in decay of plant materials. They commonly cause stored grains to heat and decay and, under certain condition, invade grains in the field (Jacobsen *et al*., 1993).

Aflatoxins are produced by *A. flavus* and *A. parasiticus* in both field and storage. Infection is most common after the kernels have been damaged by insects, birds, mites, hail, early frost, heat and drought stress, windstorms and other unfavourable weather (Jacobsen *et al*., 1993).

Aflatoxins contamination can occur in a wide variety of feedstuffs including corn, sorghum, barley, rye, wheat, peanuts, soya, rice,

cottonseed and various derivative products made from these primary feedstuffs (Busby and Wogan, 1979).

Toxigenic *A. flavus* isolates generally produce only aflatoxins B_1 and B_2, whereas *A. parasiticus* isolates generally produce aflatoxins B_1, B_2, G_1 and G_2 (Davis and Diener, 1983).The formation of aflatoxins is influenced by physical, chemical and biological factors. The physical factors include temperature and moisture. The chemical factors include the composition of the air and the nature of the substrate. Biological factors are those associated with the host species (Hesseltine, 1983).

The fungi which produce aflatoxins can be grouped into 3 classes according to their moisture requirements. The first class contains the field fungi which need 22-25% moisture. The second includes storage fungi which need 13-18% moisture and the third, advanced decay fungi, require over 18% moisture (Christensen, 1965).

Specific nutrients, such as minerals (especially zinc), vitamins, fatty acids, amino acids and energy source (preferably in the form of starch), are required for aflatoxins formation (Wyatt, 1991). Large yield of aflatoxins are associated with high carbohydrate concentrations, such as are found in wheat and rice and to a lesser extent in oilseeds such as cottonseed, soyabean and peanuts (Diener and Davis, 1968).

The limiting temperatures for the production of aflatoxins by *A. flavus* and *A. parasiticus* are reported as 12 to 41°C, with optimum production occurring between 25 and 32°C (Lillehoj, 1983). Synthesis of aflatoxins in feeds are increased at temperatures above 27°C (80 F), humidity levels greater than 62% and moisture levels in the feed above 14% (Royes and Yanong, 2002).

Aflatoxin B_1 production is stimulated by higher temperatures relative to aflatoxins G_1. Optimal AFB_1 production occurred between 24-28°C whereas 23°C is optimal for AFG_1 formation. Low temperatures (8-10°C) induce production of approximately equal amounts of aflatoxins B and G, however, total production is lowered and more time required (Weidenborner, 2001).

Chemistry and natural occurrence:

Aflatoxins were discovered back in 1960 after the outbreak of the turkey "X" disease, in England. This resulted in more than 100.000 deaths of young turkeys and 20.000 ducklings, pheasants and partridge poults. The cause was found to be a feed containing Brazilian peanuts, which was infested heavily with *A. flavus*. After much analysis of this feed, thin layer chromatography revealed that a series of fluorescent compounds, were responsible for this outbreak (Jacobsen *et al*., 1993; Rustom, 1997; Devero, 1999).

The toxic material derived from the fungus *A. flavus* was given the name "aflatoxin" in 1962 (Sargeant *et al*., 1963). Initially, two toxic components of aflatoxin were identified on thin layer chromatography plates and were named AFB and AFG due to their blue or green fluorescence under ultraviolet light, respectively (Sargeant *et al*., 1963).

In 1963, Asao *et al*.; Van Dorp *et al.* and Van der Zijden characterized the chemical and physical nature of the aflatoxins B_1, B_2, G_1 and G_2. Chemically, aflatoxins are difurocoumar-olactones (difurocoumarin derivatives). Their structure consists of a bifuran ring fused to a coumarin nucleus with a pentenone ring (in B and M aflatoxins), or a six-membered lactone ring (in G aflatoxins, (Buchi and Rae, 1969). The four compounds are separated by the color of their fluorescence under long wave (Devero,

1999) ultraviolet illumination (B=blue, G= green). Two other aflatoxins M_1 and M_2 were isolated from urine and milk and identified as mammalian metabolites of AFB_1 and AFB_2 (Patterson *et al.,* 1978).

Other metabolites B_{2a}, aflatoxicol, aflatoxicol H_1 and aflatoxins P_1 and Q_1 have been identified (FDA, 1979). Although approximately 20 aflatoxins have been identified, only 4 of them (B_1, B_2, G_1 and G_2) occur naturally. Of the aflatoxins present in food AFB_1, AFG_1 and AFM_1 are of primary importance and, together with aflatoxicol, present possible health concerns (Weidenborner, 2001). Although aflatoxins B_1, B_2 and G_1 are common in the same food sample, AFB_1 predominates (60-80% of the total aflatoxin content). Generally AFB_2, AFG_1 and AFG_2 do not occur in the absence of AFB_1. In most cases AFG_1 is found in higher concentrations than AFB_2 and AFG_2 (Weidenborner, 2001).

Mechanism of action:

AFB_1 is the most potent hepatocarcinogen known for the rat and rainbow trout and is also capable of inducing liver cancer in other animal species (Hsieh, 1985). AFB_1 can cause malignant hepatocellular carcinomas at amounts as low as 1 ppb in the diet of trout (Cheeke and Shull, 1985). This makes it one of the most abundant, most toxic and the most potent naturally occurring carcinogenic substance known (Jones *et al.,* 1994).

The carcinogenicity and mutagenicity of aflatoxins B_1 G_1 and M_1 are considered to arise as the result of the formation of a reactive epoxide at the 8, 9-position of the terminal furan ring and its subsequent covalent binding to nucleic acid (Chrevatidis *et al.,* 2003). Aflatoxins act, after bioactivation in the liver by binding of biological molecules such as essential enzymes, blockage of RNA polymerase and ribosomal translocase (inhibiting protein synthesis) and formation of DNA adducts (Angsubhakorn *etal.,* 1981; Hsieh and Atkinson, 1990). AFB_1-epoxide can bind covalently to various proteins, which may affect structural and enzymatic protein functions (Viviers and Schabort, 1985).

Although the liver is known to be the target organ of AFB_1, respiratory exposure to AFB_1 contaminated dust has been linked with increased incidence of tumor in the respiratory tract of animals and humans. Biodegradation of AFB_1 by lung cells and by nasal mucosal epithelial cells, with subsequent formation of B_1-DNA adducts has been reported (Daniels and Massey, 1992; Tjalve *et al*., 1992).

Rabbit lung microsomes have been shown to contain a high proportion of cytochrome P450 isoforms that are efficient in the activation of AFB_1 (Daniels and Massey, 1992). Bovines olfactory mucosa has high B_1 bioactivation capacity and it has been suggested that AFB_1 plays a role in the etiology of nasal tumors in cattle (Tjalve *et al*., 1992). Occupational exposure of aflatoxins through respiration was associated with an unusual increased incidence of lung cancer in Dutch workers (Autrup *et al*., 1993).

Since AFB_2 is not readily activated in rats, its carcinogenic potential is reduced by more than 150 times. It is activated in the duck liver by 2,3 desaturation from AFB_1. This desaturation process does not occur in rodents or human liver (Roebuck *et al*., 1978; FDA, 1979).

Absorption and distribution:

Because aflatoxins are very liposoluble compounds, they are readily absorbed from the site of exposure (usually the gastrointestinal tract) into blood stream (Leeson *et al*., 1995).

Absorption of AF from the respiratory system has been reported in workers at feed mills (Autrup *et al*., 1993), although there have been no studies to determine the quantitative importance of this route of absorption of aflatoxin in poultry.

When AF ingested by animals, it is readily absorbed via the gastrointestinal tract into the portal blood and is carried to the liver where it is metabolized. In the liver cells AFB is converted to classes of metabolites that may be transmitted to edible animal products. There are free or unconjugated primary metabolites of B_1, water-soluble conjugates of these metabolites, metabolites that are covalently bound to cellular macromolecules and degradation products of these B_1 adducts (Hsieh, 1983).

A portion of B_1 is activated and bound to liver tissues. Some water-soluble conjugates of B_1 metabolites are excreted into the bile and subsequently the feces. Other water-soluble conjugates and degradation products of B_1 macromolecule adducts and the uncojugated B_1 metabolites are excreted into the general circulatory blood for systemic distribution into milk or eggs and edible tissues (Hsieh, 1983 ; Eaton and Groopman, 1994).

In the liver cells, B_1 altered by cytoplasmic reductase to form aflatoxicol and by microsomal mixed-function oxidase system to form aflatoxins M_1, Q_1, P_1 and B_1 -epoxide (the most toxic and carcinogenic derivative). All of which are less toxic than B_1 and are subject to conjugation with other molecules and rapid elimination from the body (Campbell and Hayes, 1976)

Elimination:

Using radiolabelled aflatoxin in chickens has shown that the aflatoxin and its metabolites are excreted mainly through bile and to a lesser extent the kidney and gastrointestinal tract (Leeson *et al*., 1995). White Leghorn hens have been shown to excrete 28% of the aflatoxin during the first 24 hours after oral dosing and elimination of 70% within 7 days (Wyatt, 1991). Aflatoxin is also cleared form the liver of cattle over 7 days of withdrawal during which aflatoxin-free feed is provided (Helferich *et al*., 1986).

Aflatoxin B_1 is metabolized more slowly by liver tissues in sheep than in mouse, goat, guinea pig, rabbit and golden hamster. Sheep and the white rock cockerels demethylate AFB_1 poorly, sheep and dogs produce AFM_1 in comparatively large amounts (FDA, 1979).

Residues:

Aflatoxins tend to infiltrate most of the soft tissues and fat depots of the chicken (Leeson *et al*., 1995). One day after the administration of a single oral dose of ^{14}C-labelled AFB_1 to laying hens, the highest concentration of ^{14}C activity was detected in the liver, followed by muscle, pancreas, skin, adipose tissue, lungs and spleen (Sawhney *et al*., 1973a, b). In another study using ^{14}C-labelled aflatoxin, Harland and Cardeihac (1975) determined that the liver, kidney and bone marrow of chickens concentrated aflatoxins more readily than did brain, muscles or body fat.

Free and conjugated AFB1 and AFM1 were the principal tissue residues although Ro was detected in some samples (Gregory *et al*., 1983). AFM_1 is secreted in the milk of cows receiving dietary AFB_1 (Veldman *et al*., 1992). Although no evidence of AFM_1 excretion in hen's eggs has been reported, other aflatoxin metabolites can be excreted with the egg (Leeson *et al*., 1995). The aflatoxin residues in eggs has been B_1 rather than any of its known metabolites (Rodricks and Stoloff, 1977).

Sawhney *et al.* (1973 b) gave oral dose of radiolabelled aflatoxins and found different concentrations of radioactivity in all components of the egg and edible parts of the carcass. Aflatoxins or metabolites were detected in all components of the egg as early as 10 hours after ovulation and 14 hours after oviposition. The concentration of label decline in albumin after 48 hour, while levels in the yolk and shell membrane increased.

Transmission of B_1 residual into eggs requires a level of B_1 in feed considerably higher than the level that produce M_1 in milk (Rodricks and Stoloff, 1977). Lotzsch and Leistner (1977) found that delectable residues in eggs occurred only when laying hens are exposed to feed containing more than 1000 ppb B_1. While, Jacobson and Wisman (1974) recorded that the carry over of AFB_1 from layer feed to eggs was also demonstrated in hens where dietary levels of 100-400 ppb AFB_1 resulted in AFB_1 levels of 0.2 to 3.3 ppb in eggs. Despite the low levels of B_1 in eggs compared with the level of M_1 in milk, the high carcinogenic potency of B_1 makes its concentration in eggs a problem of concern (Hsieh, 1983).

In the lactating cow, AFM_1 is produced via hydroxylation of the fourth carbon in the AFB_1 molecular. AFM_2 results from hydroxylation of the fourth carbon in the AFB_2 molecule. Other aflatoxins of the M series found in milk include GM_1, GM_2, M_{2a} and GM_{2a}. They are hydroxylated derivatives of aflatoxins G_1, G_2, B_{2a} and G_{2a}, respectively (Schabort and Steyn 1969).

In animal species, ratios of aflatoxins in feeds and tissues are very low (ranging from 500: 1 to 14.000:1, excluding liver), particularly when compared (FSIS, 1998) to milk (70: 1). The concentration of AFM_1 in milk increases proportionally with the amount of AFB_1 in the diet of the lactating cow. When ingestion is continuous, milk concentrations will increase until an equilibrium with intake is established. Recent studies indicate that a greater percentage of AFB_1 is secreted in milk as AFM_1 (58:1 to 75:1), than was earlier (Harris and Staples 1992) reported (300:1).

High producing cows converted AFB_1 to AFM_1 more efficiently than did low producing cows. The ratio of dietary AFB_1 to milk AFM_1 in such cows approached the range of 66:1 to 75:1 (Frobish *et al.,* 1986; Price *et al.,* 1985). However the final concentration of AFM_1 in milk was similar in both groups due to dilution by the greatest milk production in high-producing cows (Frobish *et al.,* 1986).

The present actionable FDA guide lines for AFM_1 in milk is 0.5 ppb and for AFB_1 in feed of lactating cows is 20 ppb. According to the average transfer value of 66:1 obtained from [(58+75)/2], a concentration of 20 ppb AFB_1 in feed would result on average of 0.30 ppb AFM_1 in milk (20/66) which is below the legal maximum of 0.5 ppb. A concentration of 33 ppb AFB_1 in feed would result on average of 0.5 ppb (33/66) AFM_1 in milk, thus making the milk illegal (Harris and Staples, 1992).

A-Aflatoxicosis in humans:

Human exposure conditions: The main source of human exposure to aflatoxins is contaminated food. Two pathways of the dietary exposure have been identified: (a) direct ingestion of aflatoxins (mainly B_1) in contaminated foods of plant origin such as maize and nuts and their products, (b) ingestion of aflatoxins carried over from feed into milk and milk products including cheese and powdered milk, where they appear mainly as aflatoxin M_1 (WHO, 1979).

In addition to the carry-over into milk, residues of aflatoxins may be present in the

tissues of animals that consume contaminated feed (WHO, 1979). Aflatoxin residues have been found in animal tissues, eggs and poultry following the experimental ingestion of aflatoxin-contaminated feed (Rodricks and stoloff, 1977). Contamination of milk, egg and meat can result from animal consumption of mycotoxin-contaminated feed. Aflatoxins, ochratoxin and some trichothecences have been given considerable attention, because they are either carcinogenic or economic concern in animal health (CAST, 1989).

Aflatoxin contaminated corn and cottonseed meal in dairy rations have resulted in AFM_1 contaminated milk and milk products, including dry milk, cheese and yogurt (CAST, 1989). Natural occurrence of mycotoxins in cheese as a result of mold growth on the cheeses, also has been reported (Northolt *et al.,* 1980; Bullerman, 1981 ; Leistner, 1984).

Estimates of aflatoxin intake were provided to the European Union SCOOP project by 9 countries. The indicators of intake ranged from 2 to 77 ng/person/day for AFB_1 and from 0.4 to 6 ng/person/day for AFM_1. The USFDA estimated intakes in 1980, using data from the national compliance program for maize, groundnut and milk products using Monte Carlo stimulation procedures. The intake was 18 ng/person/day for total aflatoxins and 44 ng/ person/ day for AFM_1 (WHO, 1998).

Aflatoxin M_1 is believed to be associated with casein (protein) fraction of milk. Cream and butter contain lower concentrations of M_1 than the milk from which these products are made, while, cheese contains higher concentrations of M_1 about 3-5 times the M_1 in the original milk (Kiemeier and Buchner, 1977; Stoloff, 1980; Brackett and Marth, 1982).

Acute toxicity: Reports of acute aflatoxicosis in humans have been recorded from several parts of the world. Groups in Thailand, New Zealand, Czechoslovakia and United states have demonstrated aflatoxins in the livers of patients dying of Reye's syndrome, the lesions of which resemble closely the acute fatty liver produced in the monkey and other animals by aflatoxins (Haddad, 1990). Clinically, the main features of this syndrome are vomiting, convulsions and coma. Hypoglycaemia and elevated serum transaminases are the most constant biochemical abnormalities. Fatty degeneration in the liver and kidneys, and cerebral edema are the major and autopsy findings (Angsubhakorn, 2000).

In 1967, there was an outbreak of apparent poisoning of 26 persons in Taiwan rural villages. The victims had consumed moldy rice for up to 3 weeks. They develop the following signs: edema of the legs and feet, abdominal pain and vomiting as well as palpable liver, but no fever (Ling *et al*., 1967).

The three fatal cases were children between 4 and 8 years. Autopsies were not done, and the cause of death could not be established. In a retrospective analysis of the outbreak, a few rice samples from affected households were assayed for aflatoxins. Two of the samples contained up to 200 ppb aflatoxin B_1 (Ling *et al.,* 1967).

In 1974 an outbreak of aflatoxicosis in India was linked to moldy corn containing aflatoxin and affecting, humans and dogs. The disease was characterized by: high fever, high colored urine, vomiting, edema of feet, Jaundice, rapidly developing ascitis, portal hypertension and a high mortality rate. Of the 990 patients examined, there were at least 97 fatalities, with death in most instances due to gastrointestinal haemorrhage. The disease was confirmed to the very poor, who were forced by economic circumstances to consume badly molded corn containing aflatoxins between 6.25 -15.6 ppm, an average daily intake per person of 2-6 mg of

aflatoxins (Krishnamachari *et al.,* 1975a and 1975b; Keeler and Tu, 1983).

Cases of children disease may also be linked with acute aflatoxin ingestion in Northeast Thailand, where there is a high aflatoxin incidence in the feed. Encephalopathy and fatty degeneration of the viscera is a common cause of death among children at rural areas, with the incidence increasing during the later part of the rainy season. The disease was characterized by vomiting, convulsions, coma and death with cerebral edema and fatty involvement of the liver, kidney and heart (Shank, 1977; Van Rensburg, 1977).

In Australia, encephalopathy and fatty degeneration of the viscera in children is also referred to as Reye's syndrome described in 1963. the features of the illness include, coughing, rhinorrhea, sore throat or earache, associated the onset of the symptoms, disturbed consciousness, fever, convulsions, vomiting, disturbed respiratory rhythm, altered muscle tone and altered reflexes. At necropsy, there was cerebral edema, a slightly enlarged, firm yellow liver and a slightly widened renal cortex (Keeler and Tu, 1983).

In 1982, an acute hepatitis was reported in Kenya. There were 12 of 20 cases who died with malaise, abdominal discomfort, with subsequent appearance of dark urine and jaundice. Local dogs who shared the food were affected, with many deaths. Stored grain appeared to be the cause of the outbreak. Aflatoxin was detected in two liver samples (39 and 89 ppb). Histologically, there was centrolobular necrosis (Angsubhakorn, 2000).

In October 1988, 13 Chinese children died of acute hepatic encephalopathy in the northwestern state of peak in peninsular Malaysia (Lye *et al.,* 1995). Symptoms include, vomiting, hematemesis, fever, seize, diarrhea, abdominal pain and liver dysfunction with increased AST and ALT levels greater than 100 IU/liter. Epidemiological investigations determined that the children had eaten a Chinese noodle (Joh see fun), before they died.

Chronic toxicity: Long exposure to aflatoxins in the diet increases risk with a synergistic effect from increased alcohol consumption. Aflatoxins have been implicated as potential factors in the increased incidence of human gastrointestinal and hepatic neoplasms in Africa, The Philippines and China (CAST, 1989).

Af1atoxin B_1 has also been implicated as a cause of human hepatic cell carcinoma (HCC) (Jackson and Groopman, 1999). Pooled data from Kenya, Mozambique, Swaziland and Thailand, show a positive correlation between dietary aflatoxin intake (in the range of 3.5 to 222.4 ng/Kg body weight/day) and the crude incidence rate of primary liver cancer (ranging from 1.2 to 13.0 cases per 100.000 people per year) (WHO, 1979). Aflatoxin B_1 also chemically binds to DNA and caused structural DNA alterations with the result of genomic mutation (Groopman *et al*, 1985).

B-Aflatoxicosis in animals:

Toxicity and susceptibility of animals to aflatoxins: Aflatoxin B_1 can be classified as a highly toxic compound (LD_{50}, 1-50 mg/kg b.wt.) for most animal species, although it is extremely toxic (LD_{50}<1mg/kg) for some highly susceptible species such as rainbow trout, cats and ducklings (Leeson *et al.*, 1995). The toxicity of aflatoxins G_1, B_2 and G_2 is approximately 50, 20 and 10%, respectively, that of AFB_1 when tested against various animal species and mammalian cells in culture (Smith and Ross, 1991).

Animals of different species vary in their susceptibility to acute aflatoxin poisoning with LD_{50} values ranging from 0.3 to 17.9 mg/kg b.wt. (Table 1). In fact duckling liver

metabolized aflatoxin very rapidly in vitro (Patterson and Allcroft, 1970), although the species is sufficiently susceptible for day-old birds to be used widely in a sensitive bioassay for the toxin (Patterson, 1973). Studies indicated that rabbit, duckling and guinea-pig constitute a "fast metabolizing group" being apparently capable of handling an LD_{50} dose in under 12 minutes. Chick, mouse, pig and sheep fall into an intermediate group, metabolizing an LD_{50} dose in a few hours. So far, the rat is the only example of a "slow metabolizing group" in which LD_{50} dose would probably disappear from the liver over a period of days (Patterson, 1973).

Factors that influence aflatoxin-toxicity residue levels in animal species include: species and breeds of animals and poultry, levels and duration of exposure, nutrition and health of animals, age, sex and diseases, drugs and other mycotoxins (FDA, 1979).

Table (1): A comparison of single oral LD_{50} values for AFB_1 in various species.

Toxin	Animal	Age/ size	LD_{50} (mg/ kg)	Reference
AFB_1	Duckling	Day-old	0.37	Wogan, 1965
AFB_2			1.69 (84.8 ug/50 gm duckling)	Weidenborner, 2001
AFG_1			0.79	Lijinsky and Butler, 1966
AFG_2			2.5 (172.5 ug/ duckling)	Lijinsky and Butler, 1966; Applebaum *et al.* 1982
AFM_1			0.8 (16.6 ug/ duckling)	Purchase, 1967; Applebaum *et al.* 1982
AFB_1	Rabbit		0.3-0.5	Jones and Jones, 1969; Newberne and Butler, 1969
	Cat		0.55	Jones and Jones, 1969
	Pig	6-7 kg	0.62	Jones and Jones, 1969
	Turkey		0.5-1.0	Wogan, 1966 and 1969
	Dog	puppies	0.5-1.0	Newberne and Butler, 1969; Butler, 1974
	Cattle	young calves	0.5-1.0	Wogan, 1966 and 1969
	Guinea pig		1.4-2.0	Newberne and Butler, 1969; Wogan, 1966 and 1969
	Horse	young foals	2.0	Wogan, 1966 and 1969
	Sheep		2.0	Armbrecht *et al.,* 1970
	Monkey		2.2	Rao and Gehring, 1971
	Chickens		6.5-16.5	Smith and Hamilton, 1970
	Mouse		9.0	Jones and Jones, 1969
	Hamster		10.2	
	Rat, male	21 days	5.5	Bulter, 1964
	female		7.4	
	Male	100 gm	17.9	

Aflatoxin can cause oncogenesis, chronic toxicity or peracute signs depending on the species and age of animal and the dose and duration of aflatoxin exposure (Smith, 2002). All animal species are susceptible to aflatoxicosis, but outbreak occur mostly in pigs, sheep and cattle (Radostits, 2000).

Beef and dairy cattle are more susceptible to aflatoxicosis than sheep or horses. Young animals of all species are more susceptible than mature animals to the effects of aflatoxin. Pregnant and growing animals are less susceptible than young animals, but more susceptible than mature animals (Cassel *et al.,* 1988). Nursing animals may be affected by exposure to aflatoxin metabolites secreted in the milk (Jones *et al.,* 1994).

Among animals, young swine and pregnant sows, followed by calves (0.2 ppm in feed for 16 weeks caused mild liver damage), horses (0.4 to

0.6 ppm), fat pigs, mature cattle (0.66 ppm caused liver damage after 20 weeks) and sheep (Angsubhakorn *et al.,* 1981; Osweiler *et al.*, 1985). Levels over 1 ppm may cause severe organ damage and acute deaths in livestock (Smith, 2002).

1-Ruminants:

Aflatoxin ingested in the feed by cattle is physically bound to ruminal contents, and as little as 2-5% reach the intestine. Levels of AFB_1 in excess of 100 µg/kg of feed are considered to be poisonous for cattle (Radostits *et al*., 2000). The effects of aflatoxin fed to cattle depend on the level of aflatoxin in the ration, the length of feeding period and the age of animal (Jones *et al*., 1994).

Calves: Research has indicated that young calves and dairy cattle relatively susceptible to AFB1 contaminated ration. The LD_{50} dosage of AFB1 in calves has been estimated to be 0.5-1.0 mg/kg b.wt. (Table 1). Keyl *et al.* (1970) reported that 1.8 mg/kg b.wt. was the LD_{100} for young dairy calves. Lynch *et al.* (1971) reported histological liver damage at a minimum intake of 40 µg/kg for 6 weeks and they fed 100 µg/kg for this period without killing calves. Lynch (1972) required single doses of 200 to 1800 µg/kg b.wt. to kill calves. Pier *et al.* (1976) required 200 to 500 µg of AFB1/kg b.wt. for 14 days to produce severe pathological effects in calves.

Mckenzie *et al.* (1981) described a natural outbreak of acute aflatoxicosis among 3 to 9 months-old calves in Queensland during June, 1980. Affected calves had anorexia, depression, jaundice, photosensitization of unpigmented skin, submandibular edema, severe keratocojunctivitis and diarrhea with dysentery in some cases. Collapse and death followed rapidly.

Postmortem findings showed hemorrhages in subcutaneous tissues, skeletal muscles, lymph nodes, pericardium, beneath the epicardium and serosa of the alimentary tract. The liver was pale and carcass jaundiced. Histopathological examination of the liver revealed that hepatocytes were markedly enlarged, especially in the periportal areas, and occasional hepatocyte nuclei were up to 5 times the diameter of their companions. Hepatocyte cytoplasm was finely vacuolated, many of these vacuoles containing fat. Serum enzymes of hepatic origin and bilirubin were elevated.

In calves who have consumed contaminated rations for several weeks, the onset of clinical signs is rapid. The most consistent features are blindness, circling and falling down, with twitching of the ears and grinding of the teeth. Severe tenesmus and erosion of the rectum are seen in most cases, and death of some cases (Humphreys, 1988).

Dairy and beef cattle: The signs most commonly reported with acute toxicosis in cattle include anorexia, depression, dramatic drop in milk production, weight loss, lethargy, ascitis, icterus, tenesmus, abdominal pain (animals may stretch or kick at their abdomen), bloody diarrhea, abortion, hepatoencephalopathy, photosensitization and bleeding (Colvin *et al.,* 1984; Cook *et al.,* 1986; Ray *et al.,* 1986; Eaton and Groopman, 1994; Reagor, 1996). Other signs associated with acute aflatoxicosis include blindness, walking in circles, ear twitching, frothy at the mouth, keratoconjunctivitis and rectal prolapse (Radostits *et al.,* 2000).

Hepatic damage is a constant finding in acute aflatoxicosis. Lesions include fatty degeneration, megalocytosis and single-cell necrosis with increasing fibrosis, biliary

proliferation and veno-occlusive lesions as the disease progresses (Burnside *et al.,* 1957; Morehouse, 1981; Colvin *et al.,* 1984).

In additions, chronic aflatoxicosis may impair reproductive efficiency including abnormal estrous cycle (too short and too long) and abortions, induce immunosuppression and increase susceptibility to disease (Cassel *et al.,* 1988). The immunotoxic effect of AFB_1 were expressed via the cell-mediated immune system (Raisbeck *et al.,* 1991).

Other symptoms including decreased milk production, birth of smaller and healthy calves, diarrhea, acute mastitis, respiratory disorders, prolapsed rectum and hair loss are also observed in chronically exposed dairy cattle (Guthrie, 1979). High aflatoxin levels (4 ppm) can cause milk production to drop within one week while, lower levels (0.4 ppm) can cause production drop in 3 to 4 weeks (Hutjens, 1983). Another character of aflatoxin exposure in dairy cattle is the conversion of AFM_1 in milk (Price *et al.,* 1985). Experiments have shown that milk will be free of aflatoxin after 96 hours of feeding non-contaminated feed. The level of aflatoxin in the feed and milk at the stating point will influence clearance time (Lynch, 1972; Hutjens, 1983).

Due to risk of milk residues, dietary aflatoxin should be kept below 25 ppb. This level is conservative due to non-uniform distribution of aflatoxin in grain and feed, uncertainties in sampling and analysis and the potential for having more than one source of aflatoxin in the diet (Jones *et al.,* 1994).

The concentration of AFM_1 in milk seems to depend more on intake of AFB_1 than on milk yield (Vander Linde *et al.,* 1965). However, the toxin content of milk appears to increase rapidly when milk yield is reduced as a result of high toxin intake (Masri *et al.,* 1969). Rate of metabolism by the liver and rate of excretion by other routes (urine and feces) are also likely to influence the toxin level in milk (Applebaum *et al.,* 1982).

Decreased performance (i.e. rate of gain, milk production) is one of the most sensitive indicator of aflatoxicosis (Richard *et al.,* 1983). The ultimate cause of this effect is probably multifactorial, involving not only nutritional interactions, but also the compounding influences of anorexia, deranged hepatic protein and lipid metabolism and disturbances in hormonal metabolism (Raisbeck *et al.,* 1991). Aflatoxins have shown to affect rumen motility (Cook *et al.,* 1986) and rumen function by decreasing cellulose digestion, volatile fatty acid production and proteolysis (Fehr and Delag, 1970; Bodine and Mertins, 1983).

Sheep and goats: Anorexia, depression and icterus were observed in sheep and goats exposed to aflatoxin. The goats also developed a nasal discharge and a dark brown urine was noted in the sheep (Hatch *et al.,* 1971; Samarajeewa, *et al.*, 1975 ; Abdelsalam *et al.,* 1989).

Anorexia and diarrhea occurred in sheep given aflatoxin at a rate of 0.23 mg/ kg b.wt. These signs were accompanied by excessive salivation, tachypnea and pyrexia at dosages of 0.59 mg/kg or more. Heavy mucous diarrhea and dysentery were observed in sheep dosed at a rate of 1.28 to 2.0 mg/ kg. Sheep that died within 24 hours of dosing had marked centrilobular necrosis of the liver. Sheep that survived until the 7^{th} day after dosing had periportal congestion of the liver, widely dilated sinusoids and necrosis of liver cells (Armbrecht *et al.,* 1970).

Acutely intoxicated sheep with 4 mg/ kg b.wt. showed anorexia, diarrhea, excessive salivation, rumen atony, scour, rectal prolapse, fever and death (Wylie and Morehouse, 1978).

While, the clinical signs in less than 1 year-old goats give the same dose (4 mg/kg b.wt.) included anorexia, weakness, colic, depression, bleading, recumbency, shock, coma, convulsions and death. There was head pressing, swaying, falling and apparent blindness.

The nervous signs were probably due to the hyper ammonia which is known to occur with a variety of hepatotoxins. At necropsy, the livers varied from no lesion to presence of prominent liver lobules, pulmonary emphysema, pale kidneys and swollen hemorrhagic gall bladder walls. The histopathologic lesions varied from none or mild to severe centrilobular necrosis and bile duct proliferation (Wylie and Morehouse, 1978).

2-Non ruminant animals:

Horses: Horses are herbivores with a simple stomach. The large intestine has an active microbial digestive ability to allow digestion of forages. However, in the horse the small intestine which is the major site of absorption, occurs before the fermentative digestion. As a result, horses are more susceptible to mycotoxins than ruminants. Moreover, productive or working horses have a high energy requirement and require a high concentrate intake, and thus would be most susceptible to problems with mycotoxin contaminated grains (Jones *et al.,* 1994).

The first case of probable equine aflatoxicosis which a 15-year-old Arabian stallion died was reported by Green and Oehme (1976). Samples of the feed revealed AFB_1 levels of 54.4 ppb. The reported symptoms were anorexia, jaundice and rapid weight-loss just prior to death. Lesions of bile-duct hyperplasia, deposits of hemosiderin in epithelial cells of the renal tubules, and congestion of renal vessels were seen on histopathological examination. The liver was described as being enlarged, black in colour and having a firm consistency.

Another reported episode involved the deaths of 12 yearling colts on a breeding farm outside Bangkok, Thailand in 1981, after feeding stored ground corn and peanut meal containing 325 and 6500 ppb AFB_1, respectively. Reduced feed intake was the only symptom seen prior to death. Gross lesions observed at necropsy revealed swollen fatty livers, pale swollen kidneys and hemorrhagic enteritis. Microscopic examination documented various degrees of fatty liver and necrosis in the centrilobular zone, minimal to pronounced bile duct hyperplasia periportal fibrosis, inflammatory cell infiltration and bile duct stasis.

The experimental disease in ponies received 1, 2 and 4 mg aflatoxin/kg b.wt. (via gastric intubation), was characterized by elevated temperature, depression, anorexia, straining, convulsions, rectal prolapse and bloody feces prior to death (Asquith, 1979).

Clinical signs aflatoxicosis in adult male ponies experimentally treated with daily doses of aflatoxins (0.075, 0.15 and 0.3 mg/ kg b.wt.) consisted of lowered feed consumption, depression and terminal prostration (Cysewski *et al*., 1982). Deaths occurred at 16 and 17 days in horses given 0.3 mg/kg, 26 and 32 days in horses given 0.15 mg/ kg, and 37 and 39 days in those given 0.075 mg/kg. Prothrombin time, total plasma bilirubin, icteric index and plasma activity of aspartate aminotransferase were significantly increased. Gross lesions in dead ponies included generalized icterus, hemorrhages, brown to tan livers and dark coloured kidneys. Histopathologic findings were centrolobular fatty change with hepatic-cell necrosis and periportal fibrosis (Cysewski *et al.,* 1982).

Bortell *et al.* (1983) also treated weanling ponies with single doses of AFB_1 (via nasogastric intubation) of 0.5, 1, 2, 4 and 6 mg/ kg b.wt. and found that ponies received 4 and 6 mg/kg died after 34 and 46 hours, respectively; two of the four ponies that received 2 mg/ kg died with in 76 hours. The ponies died from acute aflatoxicosis exhibited visceral petechial hemorrhages, focal lesions in the liver and various degrees of hemorrhage in skeletal muscles.

Canine: Canine aflatoxicosis was first reported in 1952 by Seibold and Bailey who described a liver disease called hepatitis "x" which was observed in dogs fed moldy contaminated feed. Dogs and cats are extremely sensitive to aflatoxins. The LD_{50} of AFB_1 in dogs is 0.5-1.5 mg/kg and in cats is 0.3-0.6 mg/ kg b.wt. (Rumbeiha, 2001). Feed containing AFB_1 concentrations of 60 ppb or greater have caused outbreaks of aflatoxicosis in companion animals. As with other toxic compounds, sensitivity depends on individual susceptibility which in turn depends on age, hormonal status (pregnancy), and nutritional status, among other factors (Rumbeiha, 2001).

Dogs exposed to aflatoxin developed the typical anorexia, depression, icterus, prostration and blood in the feces, but also may have hemorrhages, melena and pulmonary edema (FDA, 1979; Liggett *et al.,* 1986; Bastianello *et al.,* 1987; Thornburg and Raisbeck, 1988). Moreover, vomiting, increased water consumption, polyuria, polydipsia, jaundice and elevation of serum liver enzymes in acute aflatoxicosis in dogs and cats (Rumbeiha, 2001).

At neuopsy, the liver is swollen, petechial hemorrhages are observed on the gums, along the gastrointestinal tract, in the lungs, pleura, epicardium and urinary bladder. The hemorrhages are associated with a yellow, reddish-yellow, or orange discoloration of the liver, icterus of the conjunctiva, oral mucosa, serous membranes and in body fat (Chaffee *et al.,* 1969; FDA, 1979; Rumbeiha, 2001). Lymphoid depletion and necrosis of the thymus, spleen and lymph nodes, gross uterine and placental hemorrhage and congestion and hemorrhage in the adrenal cortex were also reported (Newberne *et al.,* 1966).

In subacute aflatoxicosis, affected dogs and cats will present with lethargy, anorexia, polyuria, polydipsia, elevated liver enzymes and jaundice. In chronic aflatoxicosis, dogs and cats will have clinical signs similar to subacute aflatoxicosis, with prominent jaundice. Chronic aflatoxicosis may cause also immunosuppression, followed by non-specific clinical signs, including increased susceptibility to viral, bacterial, fungal or parasitic infections (Rumbeiha, 2001).

Histologically, there is severe fatty degeneration with distinct vacuolation of hepatocytes, bile canaliculi are distended with bile, and portal and central veins are congested with bile, and portal and central veins are congested in acute cases. In subacute cases, the distinct feature is bile duct proliferation and there is evidence of liver regeneration. In chronic cases, there is extensive liver fibrosis and bile duct proliferation (Rumbeiha, 2001).

Pigs: Young swine are extremely sensitive to aflatoxins but susceptibility decreased with age (Diekman *et al.,* 1992). The toxicity of aflatoxin is both-dose related and time related and age is an important factor in susceptibility (Lawlor and Lynch, 2001).

Sows and boars normally tolerate levels > 0.5 ppm in the feed for short periods but, when fed for extended periods, contamination levels in he feed should not exceed 0.1 ppm (Blaney

and Williams, 1991). Levels in excess of 0.5 ppm in the dites of lactating sows will depress growth rates in suckling pigs due to aflatoxin in milk. For growing and finishing pigs residues will build up in the liver at concentrations of even less than 0.1 ppm in the feed (Osweiler, 1992). The LD_{50} in young pigs dosage was determined to be 0.8 mg/kg b.wt. (Jones *et al.,* 1978).

The clinical syndrome in pigs include rough coat, depression, anorexia, decreased feed conversion, decreased rate of gain, weight loss, muscular weakness and shivering, tremors, bloody rectal discharge and icterus (Sisk *et al.,* 1968; Jones and Jones, 1978; Hoerr and D' Andrea, 1983; Radostits *et al.,* 2000). Aflatoxins also suppress the immune system and thus make pigs more susceptible to bacterial viral or parasitic diseases (Diekman *et al.,* 1992).

At necropsy, the livers from swine receiving toxic levels of AFB_1 in their ration vary in close from tan to pale yellow with atrophic gall bladders, the livers contain increased fibrous connective tissue with resistance to cutting. There is icterus and petechial hemorrhages on the heart and massive hemorrhage into the ileum or throughout the digestive tract. Microscopic lesions include irregular shaped cells, centrilobular congestion, karyorrhexis and pyknosis, vacuolation, disappearance of nuclei, bile duct proliferation and extensive connective tissue in the inter-and intralobular areas (FDA, 1979.).

3-POULTRY:

Aflatoxicosis have produced severe economic losses in the poultry industry affecting ducklings, broilers, layers, turkeys and quail (CAST, 1989). Susceptibility of poultry to aflatoxins varies among species, breeds and genetic lines. Comparative toxicological studies in avian species have shown that ducklings and turkey poults are the most sensitive species to aflatoxins. Goslings, quails and pheasants show intermediate sensitivity while chickens appear to be the most resistant (Lesson *et al.*, 1995). The susceptibility ranges from ducklings > turkey poults > goslings > pheasant chicks > chickens (Muller *et al.,* 1970).

Ducklings are 5 to 15 times more sensitive to the effects of aflatoxins than are laying hens, but when laying hen strains are compared, certain strains of hens may be as much as 3 times more sensitive than other strains (Jones *et al.,* 1994). In comparing sensitivity of different strains of leghorn chicks (table,2), it was found there is up to a 2.5 difference in the LD_{50} dose at 6 weeks of age (FDA, 1979).

Table (2): Sensitivity of different leghorn strains:

Strain	LD_{50} mg/ kg
A	6.5
B	7.25
C	9.25
D	9.50
E	11.50
F	16.50

In poultry, aflatoxin impairs all important production parameters including weight gain, feed intake, feed conversion efficiency, pigmentation, processing yield, egg production, and male and female reproductive performance. Some influences are direct effects of intoxication, while others are indirect, such as from reduced feed intake (Calnek *et al.,* 1997).

The direct and indirect effects of aflatoxicosis include increased mortality from heat stress (broiler breeders, Dafalla *et al.,* 1987a), decreased egg production (leghorns, Bryden *et al.,* 1980), anemia, hemorrhages and liver condemnations (Lamont, 1979), paralysis and lameness (Okoye *et al.,* 1988), impaired performance (broilers, Jones *et al.,* 1982), increased mortality rate (ducks, Bryden *et al.,* 1980), impaired ambulation and paralysis (quail, Wilson *et al.,* 1975), impaired

immunization (turkeys, Hegazy *et al.,* 1991), and increased susceptibility to infectious diseases (Bryden *et al.,* 1980 and Calnek *et al.,* 1997).

Ducks: Lethal aflatoxicosis in ducklings occurred as inappetance, reduced growth, abnormal vocalizations, feather picking, purple discoloration of legs and feet and lameness. Ataxia, convulsions and opisthotonus preceded death (Asplin and Carnaghan, 1961).

At necropsy, livers and kidneys were enlarged and pale. With chronicity, ascitis and hydropericardium developed accompanied by shrunken firm nodular liver, distention of the gall bladder and hemorrhages (Asplin and Carnagham, 1961; Calnek *et al.,* 1997), distended abdomen due to liver tumors and secondary ascitis (Hetzel *et al.,* 1984).

Microscopic lesions in the liver were fatty change in hepatocytes, proliferation of bile ductules and extensive fibrosis accompanied by vascular and degenerative lesions in pancreas and kidney (Asplin and Carnagham, 1961 and Calnek *et al.,* 1997). Bile duct hyperplasia and bile duct carcinoma are also reported by (Hetzel *et al.,* 1984) in aflatoxicated Campbell ducks.

Turkeys: The initial clinical signs reported during the outbreak of turkey "x" disease were anorexia and weight loss followed by depression, ataxia and recumbency. Affected birds died with in a week or two and at the time of death frequency had opisthotonus characterized by arched neck, head down back and legs extended backwards (Hamilton *et al.,* 1972).

Along with decreased feed conversion and weight gain, reduced spontaneous activity, unsteady gait, recumbency, anemia and death (Siller and Ostler, 1961; Wannop, 1961; Giambrone *et al.,* 1985 ; Richard *et al.,* 1987).

At necropsy, the body condition was generally good but there was generalized congestion and edema. The liver and kidney were congested, enlarged and firm, the gall bladder was full, and the duodenum was distended with catarrhal content (Siller and Ostler, 1961; Wannop, 1961; Calnek *et al.,* 1997).

Broilers: Decreased water and feed intake, weight loss, dullness, stunting, ruffled feathers, poor appearance and paleness, trembling, ataxia, lameness, paralysis of the legs and wings gasping, prostration and death, are frequency seen in experimental and natural outbreak of aflatoxicosis in broilers (Asuzu and Shetty, 1986; Okoye *et al.,* 1988; Rao and Joshi, 1993 ; Lesson *et al.*, 1995).

The most characteristic gross lesions appeared in the livers which were enlarged, pale yellow to grayish brown and had a prominent reticular pattern. Petecheal hemorrhages were observed on the surface of some livers. Gall bladders were enlarged and bile duct distended and there were blood in the intestinal lumen (Archibald *et al.*, 1962; Azuzu and Shetty, 1986). The liver, spleen and kidney were increased in size, whereas the bursa of fabricius and thymus were decreased (Smith and Hamilton, 1970; Huff and Doerr, 1980).

Lethal aflatoxicosis can cause either dark red or yellow discoloration of the liver due to congestion or fat accumulation, respectively (Slowik *et al.,* 1985). At chronicity livers became shrunken, firm and nodular and gall bladder was distended (Asplin and Carnaghan, 1961). The kidneys of affected birds appeared enlarged and congested (Tung *et al.,* 1973) and the spleen will be enlarged and mottled in appearance (Tung *et al.,* 1975a).

Histopathology of the liver revealed congestion of hepatic sinusoids, fecal hemorrhages, centro-lobular fatty cytoplasmic vacuolation and or necrosis, biliary hyperplasia

and nodular lymphoid infiltration. In the kidney, the epithelial cells of many tubules were vacuolated (Dafalla *et al.,* 1987 b). Azuza and Shetty (1986) and Okoye *et al.* (1988) observed severe degeneration of hepatocytes, dilation of central veins, bile duct proliferation and lymphocytic depletion in lymphoid organs in field outbreaks of aflatoxicosis in broilers.

Laying hens: Reduced egg production and egg weight, enlarged liver and increased liver fat are the most prominent manifestations of experimental aflatoxicosis in layers (Nesheim and Lvy, 1971; Hamilton and Garlich, 1972; Lesson *et al.*, 1995). High mortality and dramatic reduction of egg production were reported to occur during a natural outbreak (Hamilton, 1971). Egg size, egg weight and yolk as percent of total egg size are decreased (Huff *et al.,* 1975). In Japanese quail, decreased feed conversion, egg production, egg weight, hatchability and exterior and interior egg quality were detected (Sawhney *et al.*, 1973).

Reproduction and Hatchability: Aflatoxins causes delayed maturation of both males and females (Doerr, 1979; Doerr and Ottinger, 1980). Aflatoxicosis in white leghorn males resulted in decreased feed consumption, body weight, testes weight and semen volume (Sharlin *et al.,* 1980), and decreased plasma testosterone values (Sharlin *et al.,* 1981). While in broiler breeder males reduction in body weight and mild anemia with no alterations in semen characteristics were observed (Wyatt, 1991 ; Briggs *et al.,* 1974).

In mature laying hens experiencing aflatoxicosis, enlarged and fatty liver and marked decrease in egg production were observed (Hamilton and Garlich, 1972). Severe decline in hatchability was recoded in mature broiler breeder hens after consumption of aflatoxin (Howarth and Wyatt, 1976). Hatchability declines before egg production and is the most sensitive parameter of aflatoxicosis in broiler breeder hens (Howarth and Wyatt, 1976).

The immediate and severe decline in hatchability was found to arise from an increase in early embryonic mortality rather than infertility of the hens. The cause of the increased embryonic mortality is the transfer of toxic metabolites from the diet of the hen to the egg (Wyatt, 1991). The delayed response in egg production is thought to occur due to reducing synthesis and transport of yolk precursors in the liver (Huff *et al.,* 1975).

Immunosuppression: Aflatoxin induce immunosuppression and increases susceptibility of toxicated birds to bacterial, viral and parasitic infections. Immunosuppression caused by AFB_1 has been demonstrated in chickens and turkeys as well as laboratory animals (Sharma, 1993).

Aflatoxin decreases the concentrations of immunoglobulins IgM, IgG and IgA in birds (Giambrone *et al.,* 1978). The presence of low levels of AFB_1 in the feed appears to decrease vaccinal immunity and may therefore lead to the occurrence of disease even in properly vaccinated flocks (Lesson *et al*., 1995).

Thaxton *et al.* (1974) recorded reduced antibody production following injection of sheep red blood cells in chickens experiencing aflatoxicosis. Batra *et al.* (1991) found that chickens fed AFB_1 and vaccinated against Marek's disease showed a significantly higher frequency of gross and microscopical lesions of Marek's disease than did chickens fed aflatoxin-free diet.

Cell-mediated immune response and effector cell function are also affected during aflatoxicosis (Lesson *et al*., 1995). Aflatoxin decrease complement activity in chickens

(Campbell *et al.,* 1983 and Stewart *et al.*, 1985), and turkeys (Corrier, 1991). Since complement is required for normal phagocytosis, impairment in complement activity may partially explain impairment of phagocytosis in chickens experiencing aflatoxicosis (Gewurz and Suyehira, 1976 ; Wyatt, 1991).

Chang and Hamilton (1979a) demonstrated reduced chemotactic ability of leucocytes, impaired phagocytosis of heterophils and impaired cellular and serum factors required for optimal phayocytosis in aflatoxicated chickens. Although thrombocytic counts are depress by dietary aflatoxin (Mohiuddin *et al*., 1986) their phagocytic activity is not affected by aflatoxin (Chang and Hamilton, 1979b). However, other phagocytic cells (heterophils, macrophages and monocytes) were affected by dietary aflatoxin (Chang and Hamilton, 1979a). Chickens receiving aflatoxin-contaminated diets showed higher susceptibility to Marek's disease (Edds and Bortell, 1983), infectious bursal disease virus (Giambron *et al.,* 1978), congenitally acquired salmonellosis (Wyatt and Hamilton, 1975) and duodenal and cecal coccidiosis (Edds *et al.,* 1973; Southern *et al.,* 1984) than chickens receiving aflatoxin free diet.

From the aforementioned, it is postulated that aflatoxin interferes with normal function of B-and T-cells, rather than causing destruction of these cells (Wyatt, 1991). The impairment of protein synthesis caused by dietary aflatoxin could account for the lack of humoral immunity without the necessity of B-and T-cell destruction (Wyatt, 1991). Regardless the atrophy of the bursa of fabricius and thymus gland, the apparent alteration of splenic function is also of diagnostic significance and implies alteration in the immunocopetence of birds with aflatoxicosis (Richard *et al.,* 1975).

Hematological and biochemical alterations: Aflatoxin causes hematopietic suppression and anemia observed as decreases in total erythrocytes, packed-cell volume and hemoglobin (Reddy *et al.,* 1984; Huff *et al.,* 1986 ; Mohiuddin *et al.,* 1986). Total leucocytes is increased and differential leucocytic counts vary among studies with concurrent lymphopenia (Tung *et al.,* 1975a ; Lanza *et al.,* 1980), monocytoses and heterophilia (Wannop, 1961).

Aflatoxin is known to produce hemolytic anemia by decreasing the circulating mature erythrocytes. Lysis of erythrocytes will result in above-normal levels of cellular debris in circulation (Tung *et al.,*1975a) and consequently the spleen appear congested because of an unusually high concentration of inorganic iron and debris from the circulation (Wyatt, 1991).

Several biochemical parameters are affected by aflatoxin exposure. Aflatoxin decreases total serum proteins, alpha, beta and gamma globulins, with IgG being more sensitive than IgM (Tung *et al.,* 1975b). Total serum proteins contents are depressed due to reduced values of alpha and beta globulins and albumen, while gamma globulins are affected more variably (Pier, 1973).

Serum lipoproteins, cholesterol, triglycerides, uric acid and calcium are also decreased (Garlich *et al.,* 1973; Doerr *et al.,* 1983; Reddy *et al.,* 1984 ; Huff *et al.,* 1986). The activity of serum or plasma enzymes has been extensively used as a measure of aflatoxin activity in chickens. Increased activities of sorbitol dehydrogenase, glutamic dehydrogenase lactate dehydrogenase alkaline phosphatase, acid phosphatase, aspartate aminotransferase and alanine aminotransferase were reported in aflatoxicated chickens (Dafalla *et al.,* 1987b; Rao and Joshi, 1993; Lesson *et al.*, 1995). The increase in the levels of serum

enzymes measured was interpreted as a consequence of hepatocyte degeneration and subsequent leakage of enzymes (Lesson *et al*., 1995).

Aflatoxin has also shown to alter both the extrinsic and common clotting pathways in chickens. Aflatoxins causes biochemical changes in thromboplastin clotting factors V, VII and X and reduces plasma prothrombin and fibrinogen (Doerr *et al.,* 1976), and consequently increases whole blood clotting and prothrombin times (Doerr *et al.,* 1974). The elevated prothrombin time was considered to be the result of impaired hepatic synthesis of clotting factors caused by the toxication of alatoxin on the liver cells (Huff *et al.,* 1983).

The activity of some digestive enzymes, the absorption of carotenoid compounds from the gastrointestinal tract, and the metabolism of lipids can be altered by aflatoxin exposure (Lesson *et al*., 1995). Dietary aflatoxin produced a malabsorption syndrome characterized by steatorrhea, hypocarotenoidemia and decreased concentrations of bile salts and pancreatic lipase, trypsin, amylase and RNase (Osborne *et al.,* 1982). In another experiment, the specific activities of pancreatic chymotrypsin, amylase and lipase, but not trypsin were increased significantly by aflatoxin (Richardson and Hamilton, 1987).

The effect of aflatoxin on the renal function of broiler chickens were reported by Glahn (1993). Aflatoxin treated birds showed decreased fractional excretion of phosphate, total plasma calcium concentration, decreased total plasma proteins, plasma 25-hydroxyl vitamin D and plasma 1, 25-dihydroxy vitamin D.

Problems observed during slaughtering and processing:

Carcass pigmentation: Poor pigmentation and fatty livers were observed at processing (Lesson *et al*., 1995). Aflatoxin impairer carcass pigmentation in broiler chickens (Tyczowski and Hamilton, 1987) by inhibiting metabolism and deposition of pigments from the pathway involving the intestinal mucosa, serum, liver and integument rather than by enhancing pigment depletion from the skin (Schaeffer *et al.,* 1988).

Bruising: A more typical effect of aflatoxin is bruising (Wyatt, 1991). Aflatoxin increases susceptibility to bruising by increasing capillary fragility and reducing shear strength of skeletal muscle (Tung *et al.,* 1971). So that the blood vessels are ruptured and blood is released in surrounding tissues (Wyatt, 1991). A significant increase in capillary fragility appears to be caused by elevated lysosomal enzyme activity acting upon blood vessels. Normal tissue integrity is also compromised and blood coagulation is impaired due to decreases in numerous clotting factors notably prothrombin and fibrinogen (Doerr *et al.,* 1976; Doerr and Hamilton, 1981; Wyatt, 1991).

Abnormal liver: In addition the liver of birds with aflatoxicosis will be characteristically yellow in color with friable texture and infiltrated with fat (Wyatt, 1991). Livers from broiler with aflatoxicosis have been found to have fat content of 68% (Merkley *et al.,* 1987) and livers from breeder males fed high levels of aflatoxin revealed 300% increase in lipid content (Wyatt *et al.,* 1973). The synthesis of lipids in the liver is severely impaired, due to depressed activities of fatty acid synthetase and the microsomal enzyme system responsible for fatty acid elongation (Donaldson *et al.,* 1972), and lipid transport from the liver is severely decreased (Tung *et al.,* 1972; Markedly *et al.,*

1987). The net result of these alterations in the liver is a marked accumulation of liver lipid with depletion of normal fat reserves in the carcass (Wyatt, 1991).

4-Wild life:

Birds, fishes and mammals vary among species in susceptibility to aflatoxins. Birds such as bobwhite quail and wild turkey appear to be more susceptible than mammals (Horn *et al.,* 1989). It is difficult to document the extent to which wildlife species are affected because wild animals are free roaming and elusive. In many cases, predators and/or scavengers may consume dead or dying animals before the dead animals are found by humans (Stewart and Larson, 2002).

Clinical signs of aflatoxicosis in wildlife vary according to the dose received, the time period of exposure, and species of animal. Toxic effects can be divided into acute, subacute and chronic exposures (Stewart and Larson, 2002). Acute effects reflect severe liver disease. Animals may be anemic and may exhibit difficulty in breathing. Sudden death with no clinical signs may occur. Subacute effects may allow animals to live for a longer period of time. These animals have yellow eyes, mucous membranes, or yellowed skin along with abnormalities in blood clotting. Bruising, nose bleeds and hemorrhaging may be observed. Chronic effects are generally related to impaired liver function. Long-term, low-level consumption of aflatoxins may result in reduced feed efficiency, weight loss, lack of appetite, and increased receptivity to secondary infectious diseases. Lesions may occur in the liver and other organs and fluid may accumulate in the body cavity.

Fish: Fish have been found susceptible to aflatoxin and trichothecenes. Aflatoxicosis is most prevalent among fishes. The extent of lesions caused by consumption of aflatoxins depends upon the age and species of the fish. Fry are more susceptible to aflatoxicosis than adults and some species of fish are more sensitive to aflatoxins than others (Royes and Yanong, 2002). Rainbow trout are the most sensitive species to aflatoxin. Feeding trout diets containing less than 1 ppb will cause liver tumors in 20 months. (Horn *et al.,* 1989). Diet containing AFB_1 at 0.4 ppb for 15 months had a 14% chance of developing tumors. Feeding trout a diet containing 20 ppb for 8 months resulted in 58% occurrence of liver tumors and continued feeding for 12 months resulted in 83% incidence of tumors (Royes and Yanong, 2002).

Deaths quickly occur in 50% of stock if dietary levels of 500 to 1000 ppb are consumed. Warm water fishes such as channel catfish (*Ictalurs punctatus*) are much less sensitive than rainbow trout, and the level required to cause 50% mortality is approximately 30 times that of rainbow trout (Horn *et al.,* 1989). Channel catfish fed a diet containing purified AFB_1 at 10.000 ppb for 10 weeks exhibited decreased growth rate and moderate internal lesions (Royes and Yanong, 2002).

Initial findings associated with aflatoxicosis in fishes include pale gills, impaired blood clotting, poor growth rates or lack of weight gain. Prolonged feeding of low concentrations of AFB_1 causes liver tumors, which appear as pale yellow lesions and which can spread to the kidney. Increased in mortality may be observed (Royes and Yanong, 2002).

Aflatoxin can also depress the immune system indirect through their effect on enextial nutrients in the diet, making fish more susceptible to bacterial, viral and parasitic diseases. Moreover, aflatoxin can cause slow growth rate and reduced weight of the finished

product of warm-water fish (Royes and Yanong, 2002).

Current aflatoxin regulations (Action levels):

In 1986, the Codex Alimentarius Commission reported maximum tolerance limits for aflatoxin B in raw materials used for livestock feed by Denmark (50 ppb), Federal Republic of Germany (200 ppb), Italy (500 ppb), The Netherlands (l000 ppb) and France (100 ppb) (Jammali, 1987). The European Economic Community (EEC) has proposed a wide maximum aflatoxin tolerance level of 200 ppb in raw feed materials and supports the maximum level of 0.05 ppb for AFM_1 in liquid milk. This limit is also applicable to milk products, which are dried or processed, taking into account the connection caused by the drying process or the processing (CCFAC, 2001).

The Food and Drug Administration (FDA) has established human and animal feed levels. The action levels for human food are 20 ppb total aflatoxins with the exception of milk, that has an action level of 0.5 ppb AFM_1. For animal feeds, the action levels for aflatoxin is also 20 ppb (for all species), with the exception of a 300 ppb action level for corn used for finishing beef cattle, and 100 ppb in feeds used for breeding cattle, breeding swine and mature poultry.

A comprehensive survey of world wide regulations and guidelines (Tables, 3 and 4), as they existed on October 1, 1996. for several mycotoxins in various countries was published by the FAO in 1997.

There is no clear-cut safe levels for different animal species regarding resistance or tolerance to aflatoxins. The recommended level of aflatoxin in feed is 0 ppb. However aflatoxin-contaminated feed can be tolerated by some animals, particularly mature ones (Vincelli *et al.,* 2002). Based on the feeds available, those contaminated with aflatoxin should be fed at lowest level possible and the shortest period of time practical. If aflatoxin contaminated feed must be fed to cattle, follow these guidelines (Jones *et al.,* 1994):

-Creep feeds and diets for gestating and lactating beef cows should contain less than 20 ppb of aflatoxin.

-Unstressed, growing-finishing cattle in excess of 400 pounds may be fed diets containing up to 100 ppb of aflatoxin.

-Diets for stressed feeder cattle should contain no more than 20 ppb of aflatoxin. Stressful conditions include weaning, shipping, extreme heat or cold, diseases, and parasites.

-Animals destained for slaughter should receive of aflatoxin-free diets for at least 3 weeks before slaughter (Jones *et al.,* 1994).

-Dilute the contaminated feed with hay, haylage and wholesome grain.

-Avoid secondary mold formation and keep feed bunks.

-Use caution when feeding pregnant cattle.

-Increase protein levels (1 to 2%), energy (if possible), and vitamins A, D, E and K (Hutjens, 1983).

-Continued proper storage is essential so that aflatoxin levels do not continue to increase in the grain or prepared feed (Vincelli *et al.,* 2002).

Table (3): Maximum tolerated levels of aflatoxins in human foodstuffs

Commodity	Maximum tolerated Level (PPb)			Country
	B_1	B_1, B_2, G_1, G_2	M_1	
All foodstuffs	0			Poland, Rumania, Singapore
	1			Switzerland
		1		Honduras
		4		Germany
	5			Hungary, the Netherlands, Zimbabwe
		5		Australia, Austria, Cuba, Finland, New Zealand, Norway, Sweden, Switzerland
	10			France, Japan
		10		Egypt, Czech Republic, Italy, Peru, Spain, South Africa.
		15		Hong Kong
	20			Nigeria, Portugal
		20		Bahamas, Barbados, Colombia, Salvador, Thailand, Uruguay, United States of America.
	30			India
		30		Brazil, Ireland, Jordan, Sirlanka
		35		Malaysia
Baby foods	0			Argentine, Poland, Romania
		0.01	0.02	Honduras,
		2		Czech Republic
		3		Uruguay
Infant and young Children Foods	0			Nigeria
		0.01		Switzerland
		0.02		Austria
		0.05		Germany
		2	1	Czech Republic
		3		Uruguay
	5			Portugal
Infant foods (on milk basis)			0	Bulgaria
			0.01	Germany
			0.02	Honduras
			0.03	France
			0.05	The Netherlands
			0.1	Czech Republic
Milk and milk products			0	Egypt, Romania
			0.05	Austria, Belgium, France, Germany, Honduras, Ireland, The Netherlands, Sweden, Switzerland
			0.5	Brazil, Bulgaria, Czech Republic, Russia, Uruguay, United States of America
Liquid milk			0	Egypt, Romania
			0.05	Australia, Barbados, Belgium, France, Germany, Holland, The Netherlands, Sweden, Switzerland
			0.5	Bulgaria, Czech Republic, Mercado, Russia, United States of America, Mercosur (Argentina, Brazil, Uruguay and Paraguay)
			1	Nigeria
Milk powder			0	Egypt, Romania
			0.05	Argentina, France, The Netherland
			0.1	Bulgaria
			0.4	Australia
			5	Mercusor (Argentina, Brazil, Paraguay, Uruguay)
Milk products			0	Egypt, Romania
			0.5	Argentina, Austria, Bulgaria, China, Russia, USA
Cheese			0.2	The Netherlands
			0.25	Austria, Honduras, Switzerland
			0.5	Bulgaria
Casein	0		5	Russia

Butter			0.02	Austria, The Netherlands, Switzerland

Table (4): Maximum tolerated levels of aflatoxins in animal feedstuffs.

Commodity	Species of animal	Maximum tolerated level (ppb)		Country
		B_1	B_1, B_2, G_1, G_2	
All feedstuffs			50	Barbados, Romania
			20	Canada, Venezuela
	Reproducing animals		5	Cuba
	Depending on type of animal		20-50	Chile
	Cattle		50	Colombia
	Poultry		20	Colombia
	Animals and poultry		20	Egypt
			30	Jordan, Suriname
	Diary cattle, poultry		0	Mexico
		50		Nigeria
			10	Peru, Trinidad, Tobago
	Livestock		50	Philippines
	Poultry		20	
	Cattle, sheep, goats	50		Poland
		10		Salvador
	Diary cattle		10	Zimbabwe
Complete feedstuffs	Pigs, poultry (except young animals and ducks)		38	Cote d'voir
	Cattle, sheep, goats		75	
	Diary cattle		50	
	Other complete feedstuffs		10	
	Chicken	10		China
	Laying hens, fattening pigs	20		
	Pigs, poultry except young animals	20		European Union
	Cattle, sheep, goats except dairy cattle, calves, lambs	50		
	Calves, lambs	10		
	Diary cattle	5		
	Other complete feedstuffs	10		
		10		Oman, Sudan
	Poultry	20		Oman
	Pigs, poultry, dairy cows	20		Poland
	Cattle, sheep, goats	50		
	Cattle, sheep, goats (except dairy cattle, calves, lambs)	50		Sweden
	Pigs, poultry (except young animals)	20		
	Dairy cattle	1.5		
Complementary feedstuff	Pigs, poultry (except young animals)	30		European Union
	Cattle, sheep, goats (except diary animals, calves, lambs)	50		
	Other complementary feedstuffs	5		
	Complementary products for milk	10		Peru
Concentrated feeds			20	Guatemala

Concentrate supplementary feeds	**Porcine, poultry, dairy cattle**	**20**		**Salvador**

Table (4) : Cont.

Commodity	Species of animal	Maximum tolerated level (ppb)		Country
		B_1	B_1, B_2, G_1, G_2	
Raw materials:				
1-As straight feedstuffs				European Union
Peanut, copra, plumnut, maize, cotton seed, (products) peanut, oilseed		20		
Cereal products			10	Egypt
Maize			20	
			50	Costo Rica
Maize/peanut cake		50		China
Peanut products		10		Senegal
Peanut meal (export)			50	Brazil
		120		India
(import)		1000		Japan
Straight feedstuffs		50		Cote d'voir
2-As feedstuff ingredients:				
Soya bean		30		Argentina
Groundnut, copra, peanut, maize, cottonseed (products)	All animals	200		European Union
Feedstuff ingredients	Cattle/ sheep/ goats	50		Poland
Coconut meal, capok seed	Cows/ sheep/ pigs/ chickens/ ducks		100	Indonesia
Copra	Cows/ sheep/ pigs/ chickens/ ducks		1000	
Soyabean, maize bran, rice, sorghum, wheat polar	Cattle/ sheep/pigs/ ducks		50	
Fish, meat, bone meals	Cows, sheep, pigs, ducks		50	
Groundnut, sesame seed, rape seed meal			200	
Feedstuff raw materials			5	Cuba
Animal products		10		Peru
Feedstuff-ingredients	Cattle/ sheep/ goats	50		Poland
Cereals	Bovine and porcine fattening		200	Mexico
Peanut products		300		Senegal
Feedstuff ingredients		50		Sweden
	Dairy cattle	10		
Cereal grains and forages	Dairy cattle	1		
Groundnut, copra, palmkernel, cottonseed, maize and derived products		20		United Kingdom
Cotton seed meal	Beef cattle/ swine/ poultry		300	United states of America
Maize and peanut products	Breeding beef cattle/ swine/ mature poultry		100	
	finishing swine > 100 Lb		200	
	Finishing beef cattle		300	

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Ass. Univ. Bull. Environ. Res. Vol. 7 No. 1, March 2004

السموم الفطرية فى الأغذية والأعلاف
١-سموم الأفلاتوكسين

بدير ابراهيم عجاج

قسم بحوث الكيمياء والنقص الغذائى والسموم – معهد بحوث صحة الحيوان بالدقى – مركز البحوث الزراعية

السموم الفطرية هى مركبات كيميائية سامة تفرزها أنواع من الفطريات التى تنمو على الأغذية والمنتجات العلفية. تعتبر سموم الافلاتوكسين من أهم السموم الفطرية التى تسبب أضرار مباشرة للإنسان والحيوان بالاضافة الى إمكانية أفرازها فى اللبن والبيض. ويؤدى تعرض الإنسان لسموم الافلاتوكسين الى اضطرابات معوية وأعراض عصبية والتهابات وتليف وتسرطن بالكبد.

كما يؤدى تعرض الحيوان لسموم الافلاتوكسين إلى فقد الشهية، نقص إنتاج اللبن واللحم، اضطرابات معوية، ضعف الجهاز المناعى، أعراض عصبية بالإضافة الى الاجهاض والنفوق فى حالة التسمم الحاد. وتعتمد شدة الأعراض على نوع وعمر الحيوان، الجرعة التى تعرض لها الحيوان، ومدة التعرض بالإضافة الى الحالة الغذائية للحيوان. وفى الدجاج يؤدي التعرض للافلاتوكسين إلي ازدياد القابلية للإصابة بالأمراض الطفيلية والبكتيرية والفيروسية نتيجة ضعف الجهاز المناعى، بالإضافة إلى انخفاض معدل إنتاج البيض واللحم وازدياد معدل النفوق.

وقد استهدفت هذه الدراسة إلقاء الضوء على الفطريات المفرزة لسموم الأفلاتوكسين والظروف الملائمة لنمو هذه الفطريات على الأغذية والمنتجات العبفية، التمثيل الأقصى لسموم الأفلاتوكسين من ناحية امتصاصها وكيفية إفرازها فى الحيوانات والطيور وكذلك متبقياتها فى اللحوم والألبان والبيض، الأعراض الإكلينيكية والتغيرات الباثولوجية المصاحبة لتعرض الإنسان والحيوان والطيور المختلفة لسموم الأفلاتوكسين وكذلك الحدود المسموح بها بالنسبة لتركيز هذه السموم فى الأغذية والأعلاف حتى يمكن أتباع الإجراءات الوقائية ضد الفطريات المفرزة لها أو التخلص من هذه السموم بالطرق الخاصة بذلك.