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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 B₁, B₂, G₁ and G₂ 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 B₁ and B₂, whereas *A. parasiticus* isolates generally produce aflatoxins B₁, B₂, G₁ and G₂ (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 B₁ production is stimulated by higher temperatures relative to aflatoxins G₁. Optimal AFB₁ production occurred between 24-28°C whereas 23°C is optimal for AFG₁ 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 B₁, B₂, G₁ and G₂. Chemically, aflatoxins are difurocoumarolactones (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 M₁ and M₂ were isolated from urine and milk and identified as mammalian metabolites of AFB₁ and AFB₂ (Patterson *et al.*, 1978).

Other metabolites B_{2a}, aflatoxicol, aflatoxicol H₁ and aflatoxins P₁ and Q₁ have been identified (FDA, 1979). Although approximately 20 aflatoxins have been identified, only 4 of them (B₁, B₂, G₁ and G₂) occur naturally. Of the aflatoxins present in food AFB₁, AFG₁ and AFM₁ are of primary importance and, together with aflatoxicol, present possible health concerns (Weidenborner, 2001). Although aflatoxins B₁, B₂ and G₁ are common in the same food sample, AFB₁ predominates (60-80% of the total aflatoxin content). Generally AFB₂, AFG₁ and AFG₂ do not occur in the absence of AFB₁. In most cases AFG₁ is found in higher concentrations than AFB₂ and AFG₂ (Weidenborner, 2001).

Mechanism of action:

AFB₁ 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). AFB₁ 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 B₁, G₁ and M₁ 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 *et al.*, 1981; Hsieh and Atkinson, 1990). AFB₁-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 AFB₁, respiratory exposure to AFB₁ contaminated dust has been linked with increased incidence of tumor in the respiratory tract of animals and humans. Biodegradation of AFB₁ by lung cells and by nasal mucosal epithelial cells, with subsequent formation of B₁-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 AFB₁ (Daniels and Massey, 1992). Bovine olfactory mucosa has high B₁ bioactivation capacity and it has been suggested that AFB₁ 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 AFB₂ 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 AFB₁. 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 AFB₁ is converted to classes of metabolites that may be transmitted to edible animal products. There are free or unconjugated primary metabolites of B₁, water-soluble conjugates of these metabolites, metabolites that are covalently bound to cellular macromolecules and degradation products of these B₁ adducts (Hsieh, 1983).

A portion of B₁ is activated and bound to liver tissues. Some water-soluble conjugates of B₁ metabolites are excreted into the bile and subsequently the feces. Other water-soluble conjugates and degradation products of B₁ macromolecule adducts and the unconjugated B₁ 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, B₁ altered by cytoplasmic reductase to form aflatoxicol and by microsomal mixed-function oxidase system to form aflatoxins M₁, Q₁, P₁ and B₁ -epoxide (the most toxic and carcinogenic derivative). All of which are less toxic than B₁ 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 from the liver of cattle over 7 days of withdrawal during which aflatoxin-free feed is provided (Helferich *et al.*, 1986).

Aflatoxin B₁ 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 AFB₁ poorly, sheep and dogs produce AFM₁ 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 ¹⁴C-labelled AFB₁ to laying hens, the highest concentration of ¹⁴C 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 ¹⁴C-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). AFM₁ is secreted in the milk of cows receiving dietary AFB₁ (Veldman *et al.*, 1992). Although no evidence of AFM₁ 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 B₁ 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 B₁ residual into eggs requires a level of B₁ in feed considerably higher than the level that produce M₁ 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 B₁. While, Jacobson and Wisman (1974) recorded that the carry over of AFB₁ from layer feed to eggs was also demonstrated in hens where dietary levels of 100-400 ppb AFB₁ resulted in AFB₁ levels of 0.2 to 3.3 ppb in eggs. Despite the low levels of B₁ in eggs compared with the level of M₁ in milk, the high carcinogenic potency of B₁ makes its concentration in eggs a problem of concern (Hsieh, 1983).

In the lactating cow, AFM₁ is produced via hydroxylation of the fourth carbon in the AFB₁ molecular. AFM₂ results from hydroxylation of the fourth carbon in the AFB₂ molecule. Other aflatoxins of the M series found in milk include GM₁, GM₂, M_{2a} and GM_{2a}. They are hydroxylated derivatives of aflatoxins G₁, G₂, 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 AFM₁ in milk increases proportionally with the amount of AFB₁ 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 AFB₁ is secreted in milk as AFM₁ (58:1 to 75:1), than was earlier (Harris and Staples 1992) reported (300:1).

High producing cows converted AFB₁ to AFM₁ more efficiently than did low producing cows. The ratio of dietary AFB₁ to milk AFM₁ 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 AFM₁ 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 AFM₁ in milk is 0.5 ppb and for AFB₁ 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 AFB₁ in feed would result on average of 0.30 ppb AFM₁ in milk (20/66) which is below the legal maximum of 0.5 ppb. A concentration of 33 ppb AFB₁ in feed would result on average of 0.5 ppb (33/66) AFM₁ 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 B₁) 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 M₁ (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 trichothecenes 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 AFM₁ 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 AFB₁ and from 0.4 to 6 ng/person/day for AFM₁. 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 AFM₁ (WHO, 1998).

Aflatoxin M₁ is believed to be associated with casein (protein) fraction of milk. Cream and butter contain lower concentrations of M₁ than the milk from which these products are made, while, cheese contains higher concentrations of M₁ about 3-5 times the M₁ 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 B₁ (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, seizure, 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).

Aflatoxin B₁ 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 B₁ 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 B₁ can be classified as a highly toxic compound (LD₅₀, 1-50 mg/kg b.wt.) for most animal species, although it is extremely toxic (LD₅₀<1mg/kg) for some highly susceptible species such as rainbow trout, cats and ducklings (Leeson *et al.*, 1995). The toxicity of aflatoxins G₁, B₂ and G₂ is approximately 50, 20 and 10%, respectively, that of AFB₁ 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 LD₅₀ 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 LD₅₀ dose in under 12 minutes. Chick, mouse, pig and sheep fall into an intermediate group, metabolizing an LD₅₀ dose in a few hours. So far, the rat is the only example of a "slow metabolizing group" in

which LD₅₀ 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 LD₅₀ values for AFB₁ 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 <i>et al.</i> 1982	
AFM ₁			0.8 (16.6 ug/ duckling)	Purchase, 1967; Applebaum <i>et al.</i> 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 <i>et al.</i> , 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 AFB₁ 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 LD₅₀ 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 LD₁₀₀ 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 keratoconjunctivitis 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 AFB₁ 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 AFM₁ 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 AFM₁ in milk seems to depend more on intake of AFB₁ 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 7th 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, bleeding, 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 AFB₁ 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 AFB₁, 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 AFB₁ (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 within 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 LD₅₀ of AFB₁ 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 AFB₁ 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 neopsy, 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 the feed should not exceed 0.1 ppm (Blaney

and Williams, 1991). Levels in excess of 0.5 ppm in the dities 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 LD₅₀ 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 AFB₁ 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, karyorrhesis 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 poult 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 poult > 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 LD₅₀ 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 Carnaghan, 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 Carnaghan, 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 within 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 frequently 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. Petechial hemorrhages were observed on the surface of some livers. Gall bladders were enlarged and bile duct distended and there was 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 recorded 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 AFB₁ 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 AFB₁ 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 AFB₁ 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 phagocytosis in aflatoxicated chickens. Although thrombocytic counts are depressed 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 immunocompetence of birds with aflatoxicosis (Richard *et al.*, 1975).

Hematological and biochemical alterations: Aflatoxin causes hematopoietic 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 impairs 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 AFB₁ 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 (*Ictalurus 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 AFB₁ 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 AFB₁ causes liver tumors, which appear as pale yellow lesions and which can spread to the kidney. Increased mortality may be observed (Royes and Yanong, 2002).

Aflatoxin can also depress the immune system indirect through their effect on essential 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 (1000 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 AFM₁ 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 AFM₁. 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 destined 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
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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'voire
	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 dairy 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
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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	

REFERENCES:

- Abd El-Salam, E.B.; El-Tayeb, A.F.; Nor El Din, A.A. and Abdulmagid, A.M. (1989): "Aflatoxicosis in fattening sheep." *Vet. Rec.*; 124: 487-488.
- Angsubhakorn, S. (2000): "Mycotoxins and human health risks, an overview." In: Mycotoxin prevention and control in food grains. A collaborative publication of the UNDP/FAO, Regional Network Intercountry Cooperation on preharvest technology and quality control of food grains and ASEAN grain post-harvest programme, Bangkok, Thailand.
- Angsubhakorn, S.; Poomvises, P.; Romruen, K. and Newbern, P.M. (1981): "Aflatoxicosis in horses." *Am. Vet. Med. Assoc.*, 178: 274-278.
- Applebaum, R.S.; Brackett, R.E.; Wiseman, D.W. and Marth, E.H. (1982): "Aflatoxin: toxicity to dairy cattle and occurrence in milk and milk products." A review. *J. Food. Prot.*, 45 (8): 752-777.
- Archibald, R. McG.; Smith, H.J. and Smith, J.D. (1962): "Brazilian groundnut toxicosis in Canadian broiler chickens." *Can. Vet. J.*, 3: 322-325.
- Armbrecht, B.H.; Shalkop, W.T.; Rollins, L.D.; Pohland, A.E. and Stoloff, L. (1970): "Acute toxicity of AFB₁ in weathers." *Nature, Lond.*, 225: 1062-1063.
- Asao, T.; Bushi, G.; Abdul Kader, M.M.; Chang, G.B.; Wich, E.L. and Wogon, G.N. (1963): "The structure of aflatoxins B₁ and G₁" *J. Am. Soc.*, 882-886.
- Asplin, F.D. and Carnaghan, R.B. (1961): "The toxicity of certain groundnut meals for poultry with special reference to their effect on ducklings and chickens." *Vet. Res.*, 73: 1215-1219.
- Asquith, R. (1979): "Aflatoxicosis in Sheetland ponies. AJVR, manuscript presented for publication" In: FDA (1979).
- Asuzu, I.U. and Shetty, S.N. (1986): "Acute aflatoxicosis in broiler chicken in Nsuka, Nigeria." *Trop. Vet.*, 4: 79-80.
- Autrup, J.L.; Schmidt, J. and Autrup, H. (1993): "Exposure to AFB₁ in animal-feed production plant workers." *Environ. 41th Perspect.*, pp. 195-197.
- Bastianello, S.S.; Nesbit, J.W.; Williams, M.C. and Lange, A.L. (1987): "Pathological findings in a natural outbreak of aflatoxicosis in dogs." *Ondersteport J. Vet. Res.*, 54: 635-640.
- Batra, P.; Pruthi, A.K. and Sadana, J.R. (1991): "Effect of AFB₁ of the efficacy of turkey herpes virus vaccine against Marek's disease." *Res. Vet. Sci.*, 51: 115-119.
- Blancy, B.K. and Williams, K.C. (1991): "Effective use in livestock feeds of mouldy and weather damaged grain containing mycotoxins. Case histories and economic assessments pertaining to pig and poultry industries of Queensland." *Aust. J. Agric. Res.*, 42: 993-1012.
- Bodine, A.B. and Mrtens, D.R. (1983): "Toxicology, metabolism and physiological effects of aflatoxin in the bovine." Pp. 46-50. In: U.L. Diener; R.L. Asquith and J.W. Dickens Eds. *Aflatoxin and A. flavus in corn*. AAES, Auburn Univ., Alabama.
- Bortell, R.; Asquith, R.L.; Edds, G.T.; Simpson, C.F. and Aller, W.W. (1983): "Acute experimentally induced aflatoxicosis in the weanling pony." *Am. J. Vet. Res.*, 44: 2110.
- Brackett, R.E. and Marth, E.H. (1982): "Association of AFM₁ with casein." *Z. Lebensm Unters Forsch.* 174: 439.
- Briggs, D.M. Wyatt, R.D. and Hamilton, P.B. (1974): "The effect of dietary aflatoxin on semen characteristics of mature broiler breeder males." *Poult. Sci.*, 53: 2115-2119.

- Bryden, W.L.; Lloyd, A.B. and Cumming, R.B. (1980): "Aflatoxin contamination of Australian animal feeds and suspected cases of mycotoxicosis." *Aust. Vet. J.*, 56: 176-180.
- Buchi, G. and Rae, I.D. (1969): "The structure and chemistry of aflatoxins." In: Goldblatt, L.A. (Ed.), *Aflatoxins*, p. 55, Academic Press, New York.
- Bullerman, L.B. (1981): "Public health significance of molds and mycotoxins in fermented dairy products." *J. Dairy Sci.*, 64: 2439-2452.
- Burnside, J.E.; Sipple, W.L.; Forgacs, J.; Carll, W.T.; Atwood, M.B. and Doll, E.R. (1957): "A disease of swine and cattle caused by eating moldy corn. II. Experimental production with pure cultures of molds." *Am. J. Vet. Res.*, 18: 817.
- Busby, W.F. and Wogan, G.N. (1979): "Food-borne mycotoxins and alimentary mycotoxicosis" In: *Food-borne infections and intoxications*. 2nd ed., Riemann, H. and Bryan, F.L. eds., Academic press, New York: 537.
- Butler, W.H. (1974): "Aflatoxin" Pp. 1-28. In: I.F.H. Purchase (Ed.) *Mycotoxins*. Elsevier Scientific publ. Co., Amsterdam, the Netherlands.
- Calnek, B.C.; Barnes, H.J.; McDougald, L.R. and Saif, Y.M. (1997): "Diseases of poultry." 10th ed., Pp. 951-979. Mosby-Wolfe, Iowa state Univ. press, Ames, Iowa, USA.
- Calnek, B.W.; John Barnes, H.; Beard, C.W.; Reid, W.M. and Yoder, H.W. (1991): "Diseases of poultry." 9th ed. Pp.893-897.
- Campbell, M.L.Jr.; May, J.D.; Huff, W.E. and Doerr, J.A. (1983): "Evaluation of immunity of young broiler chickens during simultaneous aflatoxicosis and ochratoxicosis." *Poult. Sci.*, 62: 2138-2144.
- Campbell, T.C. and Hayes, J.R. (1976): *Toxicol. Appl. Pharmacol.*, 35: 199-222. Cited by Hsieh (1983).
- Cassel, E.K.; Barao, S.M. and Carmal, D.K. (1988): "Aflatoxicosis and ruminants." *Texas Vet. Med. Diagnostic lab, Texas collage. The national dairy database (1992) NDB, Health, Test, Hf100200. TxT.*
- CAST, Council for Agric. Sci. and Technol. (1989): "Mycotoxins economic and health risks." Task force report No. 116, Nov. 1989.
- CCFAC, Codex Committee on Food Additives and Contaminants (2001): "European Community, International Food Safety Issues." Directorate General Health and Consumer Protection.
- Chaffee, V.W.; Edds, G.T.; Himes, J.A. and Neal, F.C. (1969): "Aflatoxicosis in dogs" *Am. J. Vet. Res.*, 30: 1737-1749.
- Chang, C.F. and Hamilton, P.B. (1979a): "Impaired phagocytosis by heterophils from chickens during aflatoxicosis." *Toxicol. Appl. Pharmacol.*, 46: 459.
- Chang, C.F. and Hamilton, P.B. (1979b): "Refractory phagocytosis by chicken thrombocytes during aflatoxicosis." *Poult. Sci.*, 58: 559-561.
- Cheeke, P.R. and Shull, L.R. (1985): "Natural toxins in feeds and poisonous plant." Westport, Conn, AVI, Pp. 402-422.
- Chrevatidis, A.; Eggington, A.; Piacentini, M.; Wilson, P. and Patel, P. (2003): "Fact sheet on introduction to mycotoxins" 2. The aflatoxins. EMAN, Leatherhead food RA, leatherhead surrey KT227RY, UK.
- Christensen, C.M. (1965): "Fungi in cereal grains and their mycotoxins in foodstuffs" MIT press, Cambridge, Massachusetts.
- Colvin, B.M.; Harrison, L.R.; Grosser, H.S. and Hall, R.F. (1984): "Aflatoxicosis in feeder

- cattle." J. Am. Vet. Med. Assoc., 184: 956.
- Connaughton, D. (1989): "The threat of aflatoxicosis." J. Am. Vet. Med. Assoc., 194: 743-746.
- Cook, W.O.; Richard, J.L.; Osweiler, G.D. and Trampel, D.W. (1986): "Clinical and pathological changes in acute bovine aflatoxicosis: Rumen motility and tissue and fluid concentrations of aflatoxins B₁ and M₁." Am. J. Vet. Res., 47: 1817-1825.
- Corrier, D.E. (1991): "Mycotoxicosis: Mechanism of immunosuppression." Vet. Immunol. Immuno Pathol., 30: 73-87.
- Cysewski, S.J.; Pier, A.C.; Baetz, A.L. and Cheville, N.F. (1982): "Experimental Equine aflatoxicosis." Toxicol. Appl. Pharmacol., 65: 354-365.
- Dafalla, R.; Hassan, Y.M. and Adam, S.E.I. (1987a): "Fatty and hemorrhagic liver and kidney syndrome in breeding hens caused by AFB₁ and heat stress in the Sudan." Vet. Hum. Toxicol., 29: 222-226.
- Dafalla, R.; Yagi, A.I. and Adam, S.E.I. (1987b): "Experimental aflatoxicosis in Hybro-type chicks: Sequential changes in growth and serum constituents and histopathological changes." Vet. Human Toxicol., 29: 222-225.
- Daniels, J.M. and Massey, T.E. (1992): "Modulation of AFB₁ biotransformation in rabbit pulmonary and hepatic microsomes." Toxicology, 74: 19-32.
- Davis, N.D. and Diener, V.L. (1983): "Some characteristics of toxigenic and non toxigenic isolates of *A. flavus* and *A. parasiticus*." In: V.L. Diener; R.L. Asquith and J.W. Dickens; Ed. Aflatoxin and *A. flavus* in corn. Southern Coop. Series, Bull. 279, Craftmaster, Opelika, Ala. 112P.
- Devero, A. (1999): "Aflatoxins: The effects on human and animal health." BIOL, 4900; Fall, 1999.
- Diekman, A.; Coffey, M.T.; Purkhiser, E.D.; Reeves, D.E. and Young, L.G. (1992): "Mycotoxins and swine performance." CES, PTH-129, Purdue Univ., West Lafayette, Indian.
- Diener, U.L. and Davis, N.D. (1968): "Effect of environment on aflatoxin production in peanuts" Tropical science, 10: 22-25.
- Doerr, J.A. (1979): "Mycotoxicosis and avian hematosi." Diss. Abstr. B Sci. Eng., 4127.
- Doerr, J.A. and Hamilton, P.B. (1981): "Aflatoxicosis and intrinsic coagulation function in broiler chickens." Poult. Sci., 60: 528.
- Doerr, J.A. and Ottinger, M.A. (1980): "Delayed reproductive development resulting from aflatoxicosis in Juvenile Japanese quail." Poult. Sci., 59: 1995-2001.
- Doerr, J.A.; Huff, W.E.; Wabeck, C.J.; Chaloupka, G.W.; May, J.D. and Merkley, J.W. (1983): "Effects of low level chronic aflatoxicosis in broiler chickens." Poult. Sci., 62: 1971-1977.
- Doerr, J.A.; Huff, W.E.; Wyatt, R.D. and Hamilton, P.B. (1974): "Survey of T-2 toxin, ochratoxin and aflatoxin for their effects on the coagulation of blood in young broiler chickens." Poult. Sci., 53: 1729.
- Doerr, J.A.; Wyatt, R.D. and Hamilton, P.B. (1976): "Impairment of coagulation function during aflatoxicosis in young chickens." Toxicol. Appl. Pharmacol., 35: 437.
- Donaldson, W.E.; Tung, H.T. and Hamilton, P.B. (1972): "Depression of fatty acid synthesis in chick liver by aflatoxin." Comp. Biochem. Physiol., 41B: 843.
- Eaton, D.L. and Groopman, J.D. (1994): "The toxicology of aflatoxins." Human health, veterinary and agricultural significance. Pp. 6-8 Academic press, San Diego, Ca.

- Edds, G.T. and Bortell, R.A. (1983): "Biological effects of aflatoxins-poultry." Pp. 56-61. In: L.U.L. Diener, R.L. Asquity and J.W. Dickens Eds. Aflatoxin and *A. flavus* in corn AAES, Auburn Univ, Alabama.
- Edds, G.T.; Nair, N.P. and Simpson, C.F. (1973): "Effect of AFB₁ on resistance in poultry against cecal coccidiosis and Marek's disease." *Am. J. Vet. Res.*, 34: 819-826.
- FAO, Food and Agricultural Organization (1979): "FAO, Food and Nutrition." Paper No. 64, pp. 1-43; Rome.
- FDA, Food and Drug Administration (1979): "Conference on mycotoxins in animal feeds and grains related to animal health." June, 8, 1979, Rockville, Maryland.
- Fehr, P.M. and Delage, J. (1970): "Effect de l'aflatoxine sur les fermentations du rumen." *Can. Nutr. Diet.*, 5: 59-61.
- Frobish, R.A.; Bradley, B.D.; Wagner, D.D.; Long-Bradley, P.E. and Hairston, H. (1986): "Aflatoxin residues in milk of dairy cows after ingestion of naturally contaminated grains" *J. Food. Prot.*, 49: 731.
- FSIS, Food Safety and Inspection Service (1998): "National residue program." U.S. Dept. of Agric., Washington, DC20250.
- Garlich, J.D.; Tung, H.T. and Hamilton, P.B. (1973): "The effects of short term feeding of aflatoxin on egg production and some plasma constituents of laying hens." *Poult. Sci.*, 52: 2206.
- Gewurz, H. and Suyehira, L.A. (1976): "Manual of clinical immunology." Rose, N.R. and Friedman, H.R. Eds., American Society of Microbiologists, Washington, D.C., 36.
- Giambrone, J.J.; Diener, U.I.; Davis, N.D.; Panangala, V.S. and Hoerr, F.J. (1985): "Effects of aflatoxin on young turkeys and broiler chickens" *Poult. Sci.*, 64: 1678-1684.
- Giambrone, J.J.; Ewert, D.L.; Wyatt, R.D. and Eidson, C.S. (1978a): "Effect of aflatoxin on the humoral and cell-mediated immune systems of chicken." *Am. J. Vet. Res.*, 39: 305.
- Giambrone, J.J.; Partadiredja, M.; Eidson, C.S.; Kleven, S.H. and Wyatt, R.D. (1978b): "Interaction of aflatoxin with infectious bursal disease virus infection in young chickens." *Avian Dis.*, 22: 431.
- Glahn, R.P. (1993): "Mycotoxins and the avian kidney: Assessment of physiological function." *WLD. Poult. Sci. J.* 49: 242-250.
- Greene, H.J. and Ochme, F.W. (1976): "A possible case of equine aflatoxicosis." *Clin. Toxicol.*, 9: 251.
- Gregory, J.F.; Goldstein, S.L. and Eds, G.T. (1983): "Metabolite distribution and rate of residue clearance in turkeys fed a diet containing AFB₁." *Fd. Chem. Toxicol.*, 21: 463-467.
- Groopman, J.D.; Donahue, P.R.; Zhu, J.; Chen, J. and Wogan, G.N. (1985): "Aflatoxin metabolism in humans. Detection of metabolites and nucleic acid adducts in urine by affinity chromatography." *Proc. Natl. Acad. Sci., USA*, 82: 6492-6497.
- Guthrie, L.D. (1979): "Effect of aflatoxin in corn on production and reproduction in dairy cattle" *J. Dairy Sci.*: 62: 134.
- Haddad, L.H. (1990): "Clinical management of poisoning and drug overdose." 2nd Ed., p. 272, Philadelphia, P.A.: W.B. Saunders Company.
- Hamilton, P.B. (1971): "A natural and extremely severe occurrence of aflatoxicosis in laying hens." *Poult. Sci.*, 50: 1880-1882.
- Hamilton, P.B. and Garlich, J.D. (1972): "Failure of vitamin supplementation to

- alter the fatty liver syndrome by aflatoxin." *Poult. Sci.*, 51: 688.
- Hamilton, P.B., Tung, H.T.; Harris, J.R. and Donaldson, W.E. (1972): "The effect of dietary fat on aflatoxins in turkeys." *Poult. Sci.*, 51: 165-170.
- Harland, E.C. and Cardeilhac, P.T. (1975): "Excretion of carbon-14-labeled aflatoxin B₁ via bile, urine and intestinal contents of the chicken." *Am. J. Vet. Res.*, 36: 909-912.
- Harris, B.H. and Staples, C.R. (1992): "The problem of mycotoxins in dairy cattle rations." Copyright by the Univ. of Florida, Institute of food and Agric. Sci. (UF/IFAS).
- Hassad, L.M. (1990): "Clinical management of poisoning and drug overdose." 2nd ed P.271. Philadelphia, PA: W.B. Saunders Co.
- Hatch, R.C.; Clark, J.D.; Jain, A.V. and Mahaffey, E.A. (1971): "Experimentally induced acute aflatoxicosis in goats treated with ethyl maleate glutathione precursors or thiosulfate." *Am. J. Vet. Res.*, 40: 405-411.
- Hegazy, S.M.; Azzam, A. and Gabal, M.A. (1991): "Interaction of naturally occurring aflatoxins in poultry feed and immunization against fowl cholera." *Poult. Sci.*, 70: 2425-2428.
- Helferich, W.G.; Garret, W.N.; Hsieh, D.P. and Baldwin, R.L. (1986): "Feedlot performance and tissue residues of cattle consuming diets containing aflatoxins." *J. Anim. Sci.*, 62: 691.
- Hesseltine, C.W. (1983): "Introduction, definition and history of mycotoxins of importance to animal production. In: Interactions of mycotoxins in animal production." National Academy of Science, Washington, DC.
- Hetzel, D.J.S.; Hoffmann, D.; Van De Ven, J. and Soeripto, S. (1984): "Mortality rate and liver histopathology in four breeds of ducks following long term exposure to low levels of aflatoxins." *Sing. Vet. J.*, 8: 6-14.
- Horn, C.W.; Boleman, L.L.; Coffman, C.G.; Deton, J.H. and Lawhorn, D.B. (1989): "Mycotoxins in feed and food producing crops college state Texas." *Texas Vet. Med. Diagnostic, The National Dairy Database* (1992).
- Horr, F.J. and D'Andrea, G.H. (1983): "Biological effects of aflatoxin in swine." Pp. 51-55. In: U.L. Diener; R.L. Asquith and J.D. Dickens Eds. *Aflatoxin and A. flavus in corn* AAES, Auburna Univ. Alabama.
- Howarth, B.Jr. and Wyatt, R.D. (1976): "Effect of dietary aflatoxin on fertility, hatchability and progeny performance of broiler breeder hens" *Appl. Environ. Microbiol.*, 31: 680-684.
- Hsieh, D.P. (1983): "Metabolism and transmission of mycotoxins." *Proc. Int. Symp. Mycotoxins*, Sept. 6-8, 1981; NRC, Cairo Egypt. Pp. 151-165.
- Hsieh, D.P. (1985): "An assessment of liver cancer risk posed by AFM1 in Western World." In: Lacey, J. (ED.), *Trichothecenes and other mycotoxins.* Wiley & Sons, Chichester, pp. 521-527.
- Hsieh, D.P. and Atkinson, D.N. (1990): "Biological reactive intermediates and carcinogenicity." *Plenum press*, Pp. 525-532, New York.
- Huff, W.E. and Doerr, J.A. (1980): "Synergism between aflatoxin and OA in broiler chickens." *Poult. Sci.*, 60: 550.
- Huff, W.E.; Doerr, J.A.; Wabeck, C.J.; Chaloupka, O.W.; May, J.D. and Merkley, J.W. (1983): "Individual and combined effects of aflatoxin and ochratoxin A on bruising in broiler chickens." *Poult. Sci.*, 62: 1764-1771.

- Huff, W.E.; Kubena, R.B.; Harvey, R.H.; Corrier, D.E. and Mollenhaur, H.H. (1986): "Progression of aflatoxicosis in broiler chickens." *Poult. Sci.*, 65: 1891-1899.
- Huff, W.E.; Wyatt, R.D. and Hamilton, P.B. (1975): "Effects of dietary aflatoxin on certain egg yolk parameters." *Poult. Sci.*, 54: 2014-2018.
- Humphreys, D.J. (1988): "Veterinary toxicology." 3rd ed. P. 286, Bailliere Tindell, London.
- Hutjens, M. (1983): "Aflatoxin contaminated feed and dairy." In: J. Jacobsen, Aflatoxin 83. Texas Vet. Med. Diagnostic lab., Texas collage. The National Dairy Database (1992) NDB/ Feeding/ Text/ FA10004000-TXT.
- Jackson, P.E. and Groopman (1999): "Aflatoxin and liver cancer." *Bailleres Best Pract. Res. Clin. Gastroenterol.*, 13 (ISS4): 45-55.
- Jacobsen, B.J.; Brwon, K.L.; Shelby, R.A.; Diener, U.L.; Kemppainen, B.W. and Floyd, J. (1993): "Mycotoxins and mycotoxicosis circular ANR-767." Auburn Univeristy, Auburn, Alabama.
- Jacopson, W.C. and Wiseman, H.G. (1974): "The transmission of aflatoxin B₁ into eggs." *Poult. Sci.*, 53: 1743-1745.
- Jemmali, M. (1987): "Trade and economic implications of mycotoxins: Need of greater uniformity." Report No. 6, Working paper presented at the second joint FAO/WHO/UNEP, International Conference on Mycotxoins. Bangkok, Thailand, September 28 to October 3, 1987.
- Jones, F.T.; Beth, M.; Genter, M.M.; Hagler, W.M.; Hansen, J.A.; Mowrewy, B.A.; Poore, M.H. and Whitlow, L.W. (1994): "Understanding and coping with effects of mycotoxins in livestock feed and forage." Electronic publication No. DRO-29, NCCES, North Carolina State Univ., Raleigh, North Carolina.
- Jones, F.T.; Hagler, W.H. and Hamilton, P.B. (1982): "Association of low levels of aflatoxin in feeds with productivity losses in commercial broiler operations." *Poult. Sci.*, 61: 861-868.
- Jones, N.R. and Jones, B.D. (1969): "Aflatoxins." *Voeding*, 39: 330.
- Jones, N.R. and Jones, B.D. (1978): "Aflatoxins." *Voeding*, 30: 330.
- Keeler, R.F. and Tu, A.T. (1983): "Handbook of natural toxins." Vol. I. Pp. 308, Plant and fungal toxins. New York, Marcal Dekker, Ine.
- Keyl, A.C.; Booth, A.N.; Marri, M.S.; Gumbmann, M.R.; and Gagne, W.E. (1970): "Chronic effects of aflatoxin in farm animals-Feeding studies." *Proc. Ist. U.S. Japan Conf. on toxic Microorganisms, USDA, Washington, D.C.*: 72: 75.
- Kiemeier, F. and Buchner, M. (1977): "Zur verteilung van aflatoxin M₁ auf Molke and Bruch bei der Kasehersteilung." *Z. Lebensm. Unters. Forsch.*, 164: 87.
- Krishnamachari, K.A.; Bhat, R.V.; Nagarajan, V. and Tilak, T.B. (1975a): "Investigations into outbreak of hepatitis in parts of Western India." *Indian J. Med. Res.*, 63: 1036-1049.
- Krishnamachari, K.A.; Nagarajan, V.; Bhat, R.V. and Tilak, T.B. (1975b): "Hepatitis due to aflatoxicosis." An outbreak in Western India. *Lencet i*: 1061-1062.
- Lamont, M.H. (1979): "Cases of suspected mycotoxicosis as reported by veterinary investigation centers." *Proc. Mycotoxins Anim. Dis.*, 3: 38-39.
- Lanza, G.M.; Washburn, K.W. and Wyatt, R.D. (1980): "Strain variation in hematological response of broilers to dietary aflatoxin." *Poult. Sci.*, 59: 2686-2691.

- Lawlor, P.G. and Lynch, P.B. (2001): "Mycotoxins in pig feeds. 2: clinical aspects." *Irish Vet. J.*, 54 (4): 172-176.
- Leeson, S.; Diaz, G.J. and Summers, J.D. (1995): "Poultry metabolic disorders and mycotixns." Pp. 249-298, University Books, Guelph, Ontario, Canada.
- Leistner, L. (1984): "Toxigenic penicillia occurring in feeds and foods." In: H. Kurato and Y. Ueno (Eds), *Toxigenic fungi, their toxins and health hazard*, Elsevier Science Publishing Co., Inc., New York, pp. 162-171.
- Liggett, A.D.; Colvin, B.M.; Beaver, R.W. and Wilson, D.M. (1986): "Canine aflatoxicosis: A continuing problem" *Vet. Hum. Toxicol.*, 28: 428-430.
- Lijinsky, W. and Butler, W.H. (1966): "Purification and toxicity of AFG₁" *Proc. Sac. Exp. Biol. Med.*, 123: 151.
- Lillehoj, E.B. (1983): "Effect of environmental and cultural factors of aflatoxin contamination of developing corn kernels." In: V.L. Diener, R.L. Asquith and J.W. Dickens (Eds). *Aflatoxin and A. flavus in corn*. Southern Coop Serv. Bull. 279, Craftmaster, Opelika, Ala, 112P.
- Ling, K.H.; Wang, J.J.; Wu, R.; Tung, T.C.; Lin, C.K.; Lin, S.S. and Lin, T.M. (1967): "Intoxication possibly caused by AFB₁ in mouldy rice in shuang-chih township." *J. Formosan Med. Assoc.*, 66: 517-525,
- Lotzsch, R. and Leistner, L. (1977): "Ann. Nutr. Alim." 31: 499-508. Cited by Hsieh (1983).
- Lye, M.S.; Ghazali, A.A.; Mohan, J.; Alwin, N. and Nair, R.C. (1995): "An outbreak of acute hepatic encephalopathy due to severe aflatoxicosis in Malaysia." *Am. J. Trop. Med. Hyg.*, 53 (1): 68-72.
- Lynch, G.P. (1972): "Mycotoxins in feedstuffs and their effect on dairy cattle." *J. Dairy Sci.*, 55: 1243-1255.
- Lynch, G.P.; Shalkop, W.T.; Jacoby, N.M.; Smith, D.F. and Miller, R.W. (1971): *J. Dairy Sci.*, 54: 1688. In: Mackenzie *et al.* (1981).
- Lynch, G.P.; Todd, G.C.; Shalkop, W.T. and Moore, L.A. (1970): *J. Dairy Sci.*, 53: 63. In: Mackenzie *et al.* (1981).
- Masri, M.S.; Catcia, V.C. and Page, J.R. (1969): "AFM content of milk from cows fed known amounts of aflatoxin." *Vet. Rec.*, 84: 146-147.
- Mckenzie, R.A.; Blaney, B.J.; Connole, M.D. and Firzptrick, L.A. (1981): "Acute aflatoxicosis in calves fed peanut hay." *Austr. Vet. J.*, 57: 284-286.
- Merkley, J.W.; Maxwell, R.J.; Phillips, J.G. and Huff, W.E. (1987): "Hepatic fatty acids profiles in aflatoxin-exposed broiler chickens." *Poult. Sci.*, 66: 59.
- Mohiuddin, S.M.; Reddy, M.V.; Reddy, M.M. and Ramakrishna, K. (1986): "Studies on phagocytic activity and haematological changes in aflatoxicosis in poultry." *Indian Vet. J.*, 63: 442-445.
- Morhouse, L.G. (1981): "Mycotoxins-their toxicology and principle lesions." *Proc. U.S. Anim. Health Assoc.*, 232.
- Muller, R.D.; Carlson, C.W.; Semeniuk, G. and Harshfield, G.S. (1970): "The response of chicks, duckling, goslings, pheasants and poult to graded levels of aflatoxins." *Poult. Sci.*, 49: 1346-1350.
- Nesheim, M.C. and Ivy, C.A. (1971): "Effect of aflatoxin on egg production and liver fat in laying hens." *Cornell Nutr. Conf. Buffalo, N.Y.* 1971, Pp. 126-129.
- Newberne, P.M. and Butler, W.H. (1969): "Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals." *A review. Cancer Res.*, 29: 236.
- Newberne, P.M.; Russo, R. and Wogan, G.N. (1966): "Acute toxicity of AFB₁ in the dog." *Pathol. Vet.*, 3: 331-340.

- Northolt, M.D.; Van Egmond, H.P.; Soentoro, P. and Deijll, E. (1980): "Fungal growth and presence of sterigmatocystin in hard cheese." *J. Assoc. Off. Anal. Chem.*, 63: 115-117.
- Okoye, J.O.A.; Asuzu, I.U. and Gugnani, J.C. (1988): "Paralysis and lameness associated with aflatoxicosis in broilers." *Avian Pathol.*, 17: 731-734.
- Osborne, D.J.; Huff, W.E.; Hamilton, P.B. and Burtmeister, H.R. (1982): "Comparison of ochratoxin, aflatoxin and T-2 toxin for their effects on selected parameters related to digestion and evidence for specific metabolism of carotenoids in chickens." *Poult. Sci.*, 61: 1646-1652.
- Oswailer, G.D., *et al.*, (1985): "Clinical and diagnostic veterinary toxicology." Dubuque Iowa, Kendall, Hunt, Pp. 393-402. Cited by Smith, B.P (2002).
- Oswiller, G.D. (1992): "Mycooxins. In: Diseases of swine." 7th ed. Pp. 735-743. Edited by A.D. Leman, B.E. Straw, W.L. Mengeling, S.D.; Allaire and D.J. Taylor" Wolfe publishing, London.
- Patterson, D.S.; Galaney, E.M. and Roberts, B.A. (1978): "The estimation of AFM₁ in milk using 2-dimensional TLC" *Food and cosmetic toxicol.*, 16: 49-50.
- Patterson, D.S.P. (1973): "Metabolism as a factor in determining the toxic action of the aflatoxins in different animal species." *Fd. Cosmet. Toxicol.*, 11: 287-294.
- Patterson, D.S.P. and Allcroft, R. (1970): "Metabolism of aflatoxins in susceptible and resistant animal species." *Fd. Cosmet. Toxicol.*, 8: 43.
- Pier, A.C. (1973): "Effects of aflatoxin on immunity." *J. Amer. Vet. Med. Assoc.*, 163: 1268-1269.
- Pier, A.C.; Cysewski, S.J.; Richard, J.L.; Baetz, A.L. and Mitchell, L. (1976): "Proc. U.S., Anim. Hlth. Assoc., 80:130. In: Mackenzie *et al.* (1981).
- Price, R.L.; Paulson, J.H.; Lough, O.E.; Gingg, C. and Kurtz, A.G. (1985): "Aflatoxin conversion by dairy cattle consuming naturally contaminated whole cottonseed." *J. Fd. Prot.*, 48: 11-15.
- Purchase, I.F.H. (1967): "Acute toxicity of aflatoxin M₁ and M₂ in one-day old duckling." *Fd. Cosmet. Toxicol.*, 5: 339.
- Raisbeck, M.F.; Rottinghaus, G.E. and Kendall, J.D. (1991): "Effects of naturally occurring mycotoxins on ruminants." Pp. 647-677. In: Smith, J.E. and Henderson, R.S. (1991).
- Rao, K.S. and Gehring, P.J. (1971): "Acute toxicity of AFB₁ in monkeys" *Toxicol. Appl. Pharmacol.*, 19: 169.
- Rao, V.N. and Joshi, H.C. (1993): "Effect of certain drugs on acute induced aflatoxicosis in chicken (4 mg AFB₁/ kg b.wt.)." *Ind. Vet. J.*, 70: 344-347.
- Rasostits, O.M.; Gay, C.C.; Blood, D.C. and Hinchcliff, K.W. (2000): "Veterinary medicine." Pp. 1684-1688. W.B. Saunders Co. Ltd., London.
- Ray, A.C.; Abbitt, B.; Cotter, S.R.; Murphy, M.J.; Reagor, J.C.; Robinson, R.M.; West, J.E. and Whitford, H.W. (1986): "Bovine abortion and death associated with consumption of aflatoxin contaminated peanuts." *J. Am. Vet. Med. Assoc.*, 184: 956.
- Reagor, J.C. (1996): "Implications of mycotoxins in horses." *WEVR*, 96, Cybersteed, Ine.
- Reddy, D.N.; Rao, P.V.; Reddy, V.R. and Yadgiri, B. (1984): "Effect of selected levels of dietary aflatoxin on the performance of broiler chickens." *Indian, J. Anim. Sci.*, 54: 68-73.
- Richard, J.L.; Pier, A.C.; Stubblefiels, R.D.; Shotwell, O.L.; Lyon, R.L. and Cutlip, R.C. (1983): "Effect of feeding corn

- naturally contaminated with aflatoxin on feed efficiency, on physiology, immunologic and pathologic changes and in tissue residues in steers." *Am. J. Vet. Res.*, 44: 1294.
- Richard, L.J.; Stubblefield, R.D.; Lyon, R.L.; Peden, W.M.; Thurston, J.R. and Rimler, R.B. (1987): "Distribution and clearance of aflatoxin B₁ and M₁ in turkeys fed diets containing 50 or 150 ppb aflatoxin from naturally contaminated corn." *Avian Dis.*, 30: 788-793.
- Richard, T.L.; Thurston, J.R. and Pier, A.C. (1975): "Mycotoxin-induced alterations in immunity." In: *Microbiology*, Schlessinger, D. Ed., American Society of Microbiologist, Washington, D.C.
- Richardson, K.E. and Hamilton, P.B. (1987): "Enhanced production of pancreatic digestive enzymes during aflatoxicosis in egg-type chickens." *Poult. Sci.*, 66: 1470-1478.
- Rodricks, J.V. and Stoloff, L. (1977): "Mycotoxins in human and animal health." Pathotox publishers, park forest, south, 1L, Pp. 67-69.
- Roebuck, B.D.; Siegal, W.G. and Wogan, G.N. (1978): "In vitro metabolism of AFB₂ by animal and human liver cancer." *Res.*, 38: 999-1002.
- Royes, J.B. and Yanong, R.P. (2002): "Molds in fish feeds and aflatoxicosis." Copyright by the University of Florida, Institute of Agric. Sci. (UF/ IFAS).
- Rumbeiha, W. (2001): "Mycotoxicosis in pets. DCPAH-Newsletter" Vol. 18 No. 2.
- Rustom, I.Y.S. (1997): "Aflatoxin in food and feed. Occurrence, legislation and inactivation by physical methods." *food chemistry*, 59: 57-67.
- Samarajeewa, U.; Arseculeratne, S.N. and Tennekoon, G.E. (1975): "Spontaneous and experimental aflatoxicosis in goats." *Res. Vet. Sci.*, 19: 269-277.
- Sargeant, K.; Carraghan, R.B. and Allcroft, R. (1963): "Toxic products in groundnuts. Chemistry and origin." *Chem. And Ind.*, pp. 53-55.
- Sawhney, D.S.; Vadehra, D.V. and Baker, R.C. (1973a): "Aflatoxicosis in the laying Japanese quail." *Poult. Sci.*, 52: 485-493.
- Sawhney, D.S.; Vadehra, D.V. and Baker, R.C. (1973b): "The metabolism of ¹⁴C aflatoxins in laying hens." *Poult. Sci.*, 52: 1302-1309.
- Schabort, J.C. and Steyn, M. (1969): "Substrate and phenobarbitol inducible aflatoxin-4-hydroxylation and aflatoxim metabolism by rat liver microsomes" *Biochem. Pharmacol.*, 18: 2241-2252.
- Schaeffer, J.L.; Tyczkowski, J.K., Riviere, J.E. and Hamilton, P.B. (1988): "Aflatoxin-impaired ability to accumulate oxycarotenoid pigments during restoration in young chickens." *Poult., Sci.*, 67: 619-625.
- Seibold, H.R. and Bailey, W.S. (1952): "An Epizootic of hepatitis "X" in dogs." *J.A.V.M.A.*, 121: 201-206.
- Shank, R.C. (1977): "Environmental cancer." Pp. 291-318. In: H.F. Kraybill and M.A. Mehlman (eds.), John Wiley sons Ltd New York.
- Sharlin, J.S.; Howarth, B.Jr. and Wyatt, R.D. (1980): "Effect of dietary aflatoxin on serum characteristics of mature broiler breeder males." *Poult. Sci.*, 59: 1311.
- Sharlin, J.S.; Howarth, B.Jr.; Thompson, E.N. and Wyatt, R.D. (1981): "Decreased reproductive potential and reduced feed consumption in nature white leghorn males fed aflatoxin." *Poult. Sci.*, 60: 2701.
- Sharma, R.P. (1993): "Immunotoxicity of mycotoxins." *J. Dairy Sci.*, 76: 892-897.
- Siller, W.G. and Ostler, D.C. (1961): "The histopathology of an entero-hepatic syndrome of turkey poults." *Vet. Rec.*, 73: 134-138.

- Sisk, D.B.; Carlton, W.W. and Curtin, T.M. (1968): "Experimental aflatoxicosis in young swine." *Am. J. Vet. Res.*, 29: 207-215.
- Slowik, J.; Graczyk, S. and Madej, J.A. (1985): "The effect of single dose of AFB₁ on the value of nuclear index of blood lymphocytes and on histopathological changes in the liver, bursa of fabricious, suprarenal glands and spleen in ducklings." *Folia Histochem. Cytobiol.*, 3: 71-80.
- Smith, B.P. (2002): "Large animal internal medicine." 3rd ed. Pp. 1627-1637. Mosby, Inc. USA.
- Smith, J.E. and Hamilton, P.B. (1970): "Aflatoxicosis in the broiler chicken." *Poult. Sci.*, 49: 207.
- Smith, J.E. and Henderson, R.S. (1991): "Mycotoxins and animal foods." Pp. 553-726, CRC Press, Inc., Boca Raton, Boston, London.
- Smith, J.E. and Ross, K. (1991): "The toxigenic *Aspergilli*." In: Smith, J.E. and Henderson, R.S. (1991).
- Smith, J.W. and Hamillon, P.B. (1970): "Aflatoxicosis in broiler chicken." *Poult. Sci.*, 49: 207.
- Stewart, D. and Larson, E. (2002): "Aflatoxicosis in wildlife. Information Sheet 1582" Mississippi State Univ. Extension Service., Cooperating with U.S. Dept. of Agriculture.
- Stewart, R.G.; Skeeles, J.K.; Wyatt, R.D.; Brown, J.; Page, R.K.; Russell, I.D. and Lukert, P.D. (1985): "The effect of aflatoxin on complement activity in broiler chickens" *Poult. Sci.*, 64: 616-619.
- Stoloff, L. (1980): "Aflatoxin M₁ in perspective." *J. Food Prot.*, 43: 226.
- Thaxton, J.P.; Tung, H.T. and Hamilton, P.B. (1974): "Immunosuppression in chickens by aflatoxin" *Poult. Sci.*, 53: 721.
- Thornburg, L.P. and Raisbeck, M.F. (1988): "A study of canine hepatobiliary disease. Part 9: hemolytic disease, mycotoxicosis and pregnancy toxemia in the bitch." *Comp. Anim. Pract.*, 2: 13-17.
- Tjalve, H.; Larson, P.; Anderson, C. and Buck, L. (1992): "Bioactivation of AFB₁ in the bovine olfactory mucosa: DNA binding, mutagenicity and induction of sister chromatide exchanges." *Carcinogenesis*, 13: 1345-1350.
- Tung, H.T.; Cook, F.W.; Wyatt, R.D. and Hamilton, P.B. (1975a): "The anemia caused by aflatoxin" *Poult. Sci.*, 54: 1962-1969.
- Tung, H.T.; Donadson, W.E. and Hamilton, P.B. (1972): "Altered lipid transport during aflatoxicosis" *Toxicol. Appl. Pharmacol.*, 22: 97.
- Tung, H.T.; Smith, J.W. and Hamilton, P.B. (1971): "Aflatoxicosis and buruising in the chicken" *Poult. Sci.*, 50: 795-800.
- Tung, H.T.; Wyatt, R.D., Thaxton, P. and Hamilton, P.B. (1973): "Impairment of kidney function during aflatoxicosis" *Poult. Sci.*, 52: 873.
- Tung, H.T.; Wyatt, R.D.; Thaxon, P. and Hamilton, P.B. (1975b): "Concentrations of serum proteins during aflatoxicosis." *Toxicol. Appl. Pharmacol.*, 34: 320-326.
- Tyczkowski, J.K. and Hamilton, P.B. (1987): "Altered metabolism of carotenoids during aflatoxicosis in young chickens." *Poult. Sci.*, 66: 1184-1188.
- Van der Linde, L.A.; Van der Frens, A.M. and Van Esch, G.J. (1965): "Experiments with cows fed groundnut meal containing aflatoxin." P. 247. In: G.N. Wogan, ed. *Mycotoxins in foodstuffs*. MIT Press, Cambridge, mass.
- Van Dorp, D.A. and van der Zijden (1963): "Dihydro-aflatoxin B₁, metabolite of *A. flavus*" *Rec. Trax. Chem. Des pays. Bas.*, 82: 587-592.

- Van Rensburg, S.J. (1977): "Role of epidemiology in the elucidation of mycotoxin health risks." Pp. 699-712. In: J.V Rodricks; C.W. Hesseltine and M.A. Mehlman (eds), Mycotoxins in human and animal health. Pathotox publishers, Inc., Park Forest, South Illinois.
- Veldman, A.; Meijst, J.A.; Borggreve, G.J. and Heeres-Van Der Tol (1992): "Carry-over of aflatoxin from cows food to milk." Anim. Prod., 55: 163-168.
- Vincelli, P.; Paker, G. and McNeill, S. (2002): "Aflatoxin in corn." ID-59, UK Extension Publications of interest.
- Viviers, J. and Schabort, J.C. (1985): "AFB₁ alters protein phosphorylation in rat livers." Biochem. Biophys. Res. Commun., 129: 342-349.
- Wannop, C.C. (1961): "The histopathology of turkey "X" disease in great Britain." Avian Dis., 5: 371-381.
- Weidenborner, M. (2001): "Encyclopedia of food mycotoxins." Spronger Publisher Berlin, New York, London.
- WHO, World Health Organization (1979): "Environmental Health Criteria, Safety evaluation of certain food additives." Pp. 1-127 YR: 1979 IP: VI: 11.
- WHO, World Health Organization (1998): "Safety evaluation of certain food additives and contaminants." International Program on chemical safety, WHO, Food additive series, 40.
- Wilson, H.R.; Douglas, C.R.; Harms, R.H. and Edds, G.T. (1975): "Reduction of aflatoxin effects on quail." Poult. Sci., 54: 923-925.
- Wogan, C.N. (1965): "Chemical nature and biological effects of the aflatoxins." Bacteriol. Rev., 30: 460.
- Wogan, G.N. (1966): "Chemical nature and biological effects of the aflatoxins" Bacterial. Rev., 30: 460-470.
- Wogan, G.N. (1969): "Naturally occurring carcinogens in foods." Prog. Exptl. Tumr. Res., 11: 134-162.
- Wyatt, R.D. (1991): "Poultry." pp. 553-578, In: Smith, J.E. and Henderson, R.S. (1991).
- Wyatt, R.D. and Hamilton, P.B. (1975): "Interaction between aflatoxicosis and a natural infection of chickens with salmonella" Appl. Microbiol., 30: 870-872.
- Wyatt, R.D.; Briggs, D.M. and Hamilton, P.B. (1973): "The effect of dietary aflatoxin on mature broiler breeder males" Poult. Sci., 52: 1119-1123.
- Wylie, T.D. and Morehouse, L.G. (1978): "Mycotoxic fungi, mycotoxins and mycotoxicosis." Vol. 2, Pp. 414-427. Marcel Dekker, INC, USA.

السموم الفطرية فى الأغذية والأعلاف
١- سموم الأفلاتوكسين

بدير ابراهيم عجاج

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