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AFLATOXINS AND HUMAN HEALTH

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ABSTRACT

Aflatoxins (AF) are the secondary metabolites produced mostly by certain species of *Asperigillus flavus* and *Asperigillus parasiticus*. These toxins have closely similar structures and form a unique group of highly oxygenated, naturally occurring heterocyclic compounds. Their molecular formulae are established from elementary analyses and mass spectrometric determinations. These compounds contaminate a variety of food and feed products and play an important role in domestic animals and humans health. Also, these compounds caused many acute disease syndromes, while at lowest levels they can be carcinogenic, mutagenic, teratogenic or estrogenic and could reduce the productive performance. The performance of animals such as milk, gain, meat, egg production ....etc., were affected with aflatoxin-contaminated diets.

Aflatoxin AFB_1 and its metabolite residues, had been found in muscles and edible organs of exposed animals (that consumed AF-contaminated feeds), including liver, heart and kidney. So, humans are exposed to AF by direct consumption of contaminated animal products (meat, milk, eggs,...). It is therefore, important to keep the AF intake very low, in order to minimize the danger of AFM_1 entering the human food-chain, and that are considered potentially hazardous to human consumers. The Codex Committee For Food and Additive and Contaminate (CCFAC) has recently legislated a 0.05 µg / Kg AF limit in dairy concentrate products. Therefore, practical and least cost-effective methods to detoxify AF-contaminated feedstuffs are in great demand. Recently, many approaches were applied for the detoxification of AF such as the use of medicinal herbs mixture (MHM), anti-toxin compounds and Bentonite materials in the diet to diminish the harmful effects of AF.

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INTRODUCTION

During 1960, more than 100,000 young turkeys on poultry farms in England died in the course of a few months from an apparently new disease that was termed "Turkey X disease". It was soon found that the problem was not limited to turkeys. Ducklings and young pheasants were also affected and heavy mortality was experienced.

A careful survey of the early outbreaks showed that they all were associated with feeds namely Brazilian peanut meal. An intensive investigation of the suspect peanut meal was undertaken and it was quickly found that this peanut meal was highly toxic to poultry and ducklings with symptoms typical of Turkey X disease.

Speculations made during 1960 regarding the nature of the toxin suggested that it might be of fungal origin. In fact, the toxin was identified as the secondary metabolites which are produced mostly by certain species of *Asperigillus flavus* and *Asperigillus parasiticus* (**Habish *et al.*, 1972** and Rao *et al.*, 1965).).

This discovery has led to a growing awareness of the potential hazards of these substances as contaminants of food and feed causing illness, cancer and even death in humans and other mammals (**Groy *et al.*, 1978).**

Studies revealed that there are four major aflatoxins: B_1, B_2, G_1 and G_2 plus two additional metabolic products, M_1 and M_2, that are of significance as direct contaminants of foods and feeds. The AF M_1 and M_2 were first isolated from milk of lactating animals fed AF-contaminated diets (**Allcroft *et al.*, 1966**).

These toxins have closely similar structures and form a unique group of highly oxygenated, naturally occurring heterocyclic compounds. Their molecular formulas as established from elementary analyses and mass spectrometric determinations (**Neal et al. , 1981**). These compounds contaminate a variety of food and feed products and they play an important role in domestic animals and humans health. Aflatoxins seem to be of greatest threat to animal health and efficient livestock production (**Wogan, 1973**).

Under the Egyptian conditions, 45 isolates of *Aspergilli* were tested for the production of mycotoxins. These fungi were isolated from soil, seeds grains and air. Fifteen isolates were non toxic and induced no effect on the hatchability of eggs, while thirty isolates were toxic. Six isolates of them were of strong effect (**Moubashor et al., 1977**).

The gap in livestock feeds is considered a major problem in Egypt, because of the shortage of cereals and the limited cultivated area and the extremely continuous increased demands, while the secondary problem is the presence of mould in feedstuffs during the handling and storage with primitive bad methods.

Oil seed meals and cereal grains are considered mostly contaminated feedstuffs with AF. As a matter of fact the losses caused by fungal contamination is mainly due to the rejection of feed with visible fungal growth and as well to its probable content of aflatoxin **(Hopf, 1977).**

When these contaminated feeds are consumed by livestock at relatively high levels (greater than 500 ppb), adverse effects such as decreased feed intake and subsequently decreased feed efficiency are observed **(Lynch 1972)**. Also, these levels caused many acute disease syndromes, while at lowest levels it can be carcinogenic, mutagenic, teratogenic or estrogenic and may reduce the productive performance.

Long term feeding trial on sheep using contaminated ground nut meal (1.75 ppm AF) induced hepatic carcinoma **(Lewis et al., 1967).**

The AFB_1 and its metabolite residues, had been found in muscles and edible organs of exposed animals (that consumed AF-contaminated feeds), including liver, heart and kidney. So, humans are exposed to AF by direct consumption of contaminated animal products (meat, milk, eggs..) **(Shanon et al., 1983 and Saluankle et al., 1987).**

Chemical, physical and biological processing such as ammonia, hydrogen peroxide, diethyl-amine, clays (hydrated sodium calcium aluminu-silicate, bentonite, kaolin, has been intensively used in a wide range and applied under field conditions to decrease the concentrations of AF in animal feedstuffs and reduce the negative effects of AF(**Abdelhameed et al., 1995, Nowar et al., 2001, Soliman et al., 2003, Soliman, A. M. and M., Rashed 2006).**

FACTORS FAVORIZING AFLATOXIN PRODUCTION in foods and animal feedstuffs could be summarized in; physical (as relative humidity and moisture), temperature and time, chemical (substrate i.e. cereals, oilseeds), chemical composition of air (CO_2/O_2 ratio), biological (as fungus and insects), maturity and lastly damage and microbial inactivation(**(Calderwood and Schroeder, 1968, Diener and Davis, 1968, Shanon, 1974, Moubasshor et al., 1977).**

EFFECTS OF AFLATOXIN ON ANIMAL PERFORMANCE

Many incidents of disease outbreaks in farm animals have been reported in many countries to be associated with contaminating feed with aflatoxin or infecting with *A. flavus* mould. The toxicity of aflatoxin has been studied in cattle, sheep, goats, and other animals. The production of animals such as milk, gain, meat, egg ....etc,, were affected with aflatoxin-contaminated diets.

FEED INTAKE AND FEED EFFICIENCY

Voluntary intake by calves was reduced after daily dose of 80 µg AFB/Kg body weight /week, which means that the calves might lost some of their appetite. There was a reduction in feed intake and body weight gain in calves receiving AF. Meanwhile, adverse effects, such as decreased feed intake and a subsequent decrease in feed efficiency were reported (**Lynch *et al.*, 1979**). Studies of **Armbercht *et al.*, 1970** recognized loss of appetite and diarrhea with toxic level of 0.23 mg /Kg in weathers

MILK PRODUCTION

Aflatoxin M_1 is of particular importance since it is the toxic metabolite found in the milk of lactating animals ingested AFB_1. It has been found in milk products, such as skim milk, which in turn may be fed to other farm animals **(Senser, 1967).** The adult cattle were much resistant to the diet containing toxic groundnut meal than calves, but the lactating cows showed a significant reduction in milk yield within a few days of eating contaminated rations **(Allcroft and Lewis, 1963).**

High levels of AF do decrease the milk yield quite dramatically(8 % decrease in milk production) while, in some cases milk production of cows fed diet containing 1 ppm AF, decreased by 33 % after six weeks, and that cows fed diet containing 4 ppm

AF gave the same decrease in production, but only after two weeks on that diet **(Ralph et al., 1985).**

Cow has a comparatively low ratio of AFB_1 in feed /AFM_1 in milk of 100 .i.e. a cow ingested 100 ppb AFB_1 will produce milk containing 1 ppb AFM_1. Some investigations, reported that, lactating dairy cattle secrete 1.7 % of their total AFB_1 intake, this percent is affected by the feed ingredient containing the AF and by the level of milk production of the animals receiving the contaminated feed **(Frobish et al., 1986).**

Many investigators mentioned that Aflatoxins are transmitted from feed of lactating animals to milk. Investigations on this transfer, in general, have been limited to the detection of AFM_1 in milk. These transmission data are limited because they consider only a milk to feed percentage ratio. It is therefore, important to keep the AF intake very low, in order to minimize the danger of AFM_1 entering the human food chain, and that are considered potentially hazardous to human consumers. Because AFM_1 has a similar acute toxicity to AFB_1 and is also a very active carcinogen in some animals, it has being at least a tenth as potent as AFB_1. **(Masri et al., 1969; Purchase, 1972 and Patterson et al., 1980).**

The Codex Committee For Food and Additive and Contaminate (CCFAC, 1998) has recently legislated a 0.05 µg / kg AF limit in dairy products.

GROWTH

Although sheep and goats are generally considered resistant to AF than other species, growing lambs can be subjected to the toxic effects of AF such as depressed weight gains and death, which can be of economic importance. It can be observed that, the deleterious effect of AF on growth could occurred by either low quality or lack of protein of the daily ration **(Ostrowski-Meissner, 1983).** Cows fed 1.5 mg AF daily (0.30 ppm) in ground nut cake and nursing calves showed a reduction of growth.

Male goats, showed a decrease in live body weight gain and daily gain, when fed an AF-contaminated corn. Also, when the contaminated feeds (cottonseed meal, corn and other contaminated feed) are consumed by livestock at relatively high levels (greater than 500 ppb), less body weight gain was recorded **(Abd-El-Galil, 1993).**

BILOGICAL EFFECT OF AFLATOXIN

BLOOD:

Aflatoxin B_1 ingestion did not affect blood cells contents. The physiological effects of AF consumption include liver damage characterized by release of enzymes into the blood. Serum alkaline phosphatase is one of the enzymes level that increase in activity in response to AFB_1 feeding . On the other side, enzymes and blood compounds that, decreased, were serum urea nitrogen, serum albumin, total serum protein, lipid and vitamin A.

The features of aflatoxicosis in ruminants include blood coagulation defects, which probably involve the important activity of prothrombin **(Pier, 1992)**. However, serum glutamic-pyruvic transaminase (GPT) and liver alkaline phosphatase increased when feeding an AF contaminated diets. On the other hand, total serum proteins, albumin and globulin were unaffected by AF consumption but gamma globulins were increased in pigs receiving 750 and 1480 ppb AF **(Southern and Clwson, 1979)**.

Serum GOT, GPT and alkaline phosphatase, were significantly affected (positive indication) when ruminants ingested aflatoxins contaminated feedstuffs **(Abd-El-Galil, 1993)**.

Cows fed 1.5 mg AF daily (0.30 ppm) in groundnut cake and nursing calves showed an increase in total blood lipids and a decrease in urinary urea, while the calves showed increase in serum glutamic oxaloacetic transaminase (GOT), traces of AF were found in viscera and muscle samples from one of the treated cows **(Cast, 1989).**

Polan et al., (1974) reported that, toxins are rapidly absorbed into the blood and transferred to the milk of cows fed rations contaminated with AFB_1. This provided evidence that the toxin could enter the blood directly through the rumen epithelium and were then available for distribution to the tissues.

CLINICAL SYMPTOMS

a-CATTLE

When calves consume contaminated rations for several weeks, the onset of clinical signs is rapid. The most constant features are blindness, circling and falling down, with twitching of the ears and grinding of the teeth, Sever tenesmus and aversion of the rectum are seen in most cases. Death usually follows within two days of the onset of severe clinical signs **(Lynch et al., 1972; Patterson and Anderson, 1982 and Applebaum and Marth, 1983)**, while in non fatal cases there is a marked loss of condition, and recovery is very slow. All these previous signs were observed by many investigators. Older cattle seldom show clinical abnormalities, but there is a fall in milk yield **(Lynch et al., 1972).**

b-SHEEP AND GOATS

Sheep and goats are very resistant to poisoning by AF **(Lewis et al., 1967)**. Ewes fed on highly toxic groundnut meal for five years showed no abnormalities except lowered fertility and various neoplasms, which may or may not have been significant. This is mainly due to that most of the aflatoxin administered to sheep appears to be destroyed in the body, only 8% of the amount given being recovered in the milk , urine and feces **(Nabney et al., 1966)**. Also, with different doses of AFB_1, it was found that loss of appetite and diarrhea occurred at 0.23 mg/kg and higher, excessive salivation forming at the mouth and increased rectal temperature within 12 hr were seen at level 1.28 µg/kg, while higher dose (1.28 - 2.0 mg/kg)

caused an increase rate of respiration **(Armbrecht et al., 1970)**. Aflatoxicosis has occurred in goats fed on a concentrate mixture containing defatted grated coconut residues. Inappetance, apathy and neurological signs were seen in flock of sheep near Khartoum, Sudan, fed on groundnut oil meal contaminated with 750 µg / kg **(Suliman et al., 1987)**.

CARCASSE AND EDIBLE ORGANS (POST MORTEM)

The liver of the animal is one of the first organs affected by aflatoxins. Studies on calves fed for 6 weeks a compound feed containing 15 % Barazilion peanut meal Revealed that the livers of the animals exhibited areas of fibrosis with bile duct proliferation **(Loosmore and Markson (1961)**.

Also, **Markson, 1963** reported that when 3- 9 months old calves, consumed a compound feed contaminated Barazillian peanut meal for 6 weeks, the livers exhibited areas of fibrosis with biliary proliferation. An outbreak occurring about the same time in 1.5 -2 years old cattle, with symptoms and lesions identical to those described above.

Similar outbreak resulted in (1962), the dead cattle have the same symptoms and was also reported for calves fed maize containing 2,000 ppb aflatoxins. Some young fattening cattle died and post mortem examination of the cattle showed haemorrhage of the bladder, stomach, small and large intestines, heart and liver **(Otero, 1982)**. The poisoning of calves and old cattle by compound feed containing 2 ppm of AF revealed that the livers of animals killed after 11 weeks and central veins were partially or completely obliterated by fibrous tissue **(Allcroft and Lewis 1963)**.

Lewis et al., (1967) reported that long term feeding using contaminated groundnut meal (1.75 ppm AF) resulted in hepatic carcinoma in sheep.

Also, a dose of 1.28 - 2.0 mg/Kg had killed a Marino sheep. All animals that, died in 15 - 16 hr. had massine controlobular necrosis of the liver and sometimes had necrotic bridges extending to contiguous central veins, while, sheep that, survived until 7^{th} day after dosing had periportal congestion of the liver, widely dilated sinusoids and necrosis of liver cell **(Armbrecht et al., 1970)**. Investigation of **Lynch et al., (1979)** on daily feeding aflatoxin contaminated feed to young calves has shown pathological and clinical biochemical changes similar to those in other mammalian species. In addition, there were hemorrhage in the spleen and gall-bladder and hestological changes in the liver and kidney of cows received AFB_1, including failure of the detoxifying mechanism. Sheep fed on groundnut oil meal contaminated with 750 µg / kg, showed gross and microscopic lesions confined to the liver **(Suliman et al., 1987)**. The presence of AF in the feeds and tissues of dead sheep supported the view that the condition was due to AF poisoning.

On the other hand, a diet containing 2.6 mg of AF/Kg feed may induce aflatoxicosis in growing weathers. These results were similar to those reported for swine experimentally fed 0.75 mg of AF/Kg of feed and indicated that lambs were more sensitive to the toxic effects of AF than was previously reported **(Harvey et al., 1991)**.

RESIDUES OF AFLATOXINS IN MUSCLES AND EDIBLE TISSUES

Aflatoxin B_1 residues have been found in muscles and the edible tissues of exposed animals, including liver, heart and kidney. However, liver is considered the principle organ for AFB_1 metabolism. The active metabolites of AFB_1 would bind to proteins and nucleic acids and be retained in the liver cells. Therefore, liver tissues would contain the highest level of AFB_1 residues and serve as an indicator of animal exposure to AFB_1 and the possible presence of AFB_1 residues in other tissues **(Hsieh, 1981)**. **Southern and Clowson (1979)** fond that liver weight expressed as a percentage of final live weight were increased in pigs consumed 385 ppb in a group of treatments . Studies on the metabolism of AFB_1 in animals may shed light on conditions under which the AFB_1 detoxification mechanism in the exposed animals may be enhanced to minimize transmission of AFB_1 and AFM_1 to edible animal products **(Hsieh, 1981)**.

In the muscle tissues of pigs fed a contaminated diet, the levels of both AFB_1 and AFM_1 were about one half of the levels in the liver **(Furtado et al., 1981)**. The highest concentration found was 137 ppb in pig liver and 54 ppb in kidney **(Krogh, 1972)**.

Other tissues that may contain AFB_1 residues comparable to liver are kidney and heart. Bovine kidneys may even be better than pig liver as an indicator of AF exposure. Cattle showed an increase in total blood lipids and decreased in urine urea, while the calves showed increase in serum glutamic oxaloacetic transaminase (GOT). Traces of AF were found in viscera and muscle samples from one of the treated cows. Also, the physiological effects of AF consumption include liver damage characterized by release of enzymes into the blood **(Southern and Clawson, 1979)**.

Rapid absorption of the toxins into the blood and milk of cows fed ration contaminated with AFB_1, provided evidence that, the toxin could enter the blood directly through the rumen epithelium and were then available for distribution to the tissues. Therefore practical and least cost-effective methods to detoxify AF-contaminated feedstuffs are in great demand **(Calvet et al., 1966)**. Recently, many approaches are applied for the detoxification of AF such as the use of medicinal herbs mixture (MHM), anti-toxin compounds and bentonite materials in the diet to reduce and diminish the harmful effects of AF.

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