AFLATOXINS AND IMMUNITY : A. REVIEW

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ABSTRACT

Aflatoxins are toxic metabolites produced by *Aspergillus flavus* and *A. parasiticus*. These agents can cause severe hepatotoxicosis in many species of animals and carcinogenesis in others. Farm animals are generally sensitive to aflatoxins and ducks are among the most susceptible species. The toxins also interfere with the development of native and acquired resistance of animals to infections diseases. The effect of aflatoxins on the avian immunity is particularly described. In a tropical country like Indonesia where the toxins occur naturally in feeds, regardless of the mechanisms the immunosuppressive effect of aflatoxins is one of real economic importance to the livestock industry of Indonesia.

Aflatoksin adalah racun-racun yang dihasilkan oleh jamur-jamur *Aspergillus flavus* dan *A. parasiticus*. Racun tersebut bisa menyebabkan keracunan hati yang hebat pada banyak jenis hewan dan juga bisa menimbulkan tumor hati pada hewan. Hewan ternak pada umumnya peka terhadap aflatoksin dan itik adalah hewan yang paling peka. Racun-racun tersebut juga mempengaruhi perkembangan dari kekebalan alam maupun kekebalan perolehan terhadap penyakit-penyakit menular. Pengaruh aflatoksin pada kekebalan unggas secara khusus dibahas. Di negara tropis seperti Indonesia dimana racun-racun ini selalu mencemari pakan, pengaruh aflatoksin yang menekan sistem kekebalan adalah merupakan kerugian ekonomis yang sangat besar pada industri peternakan di Indonesia.

I. INTRODUCTION

Aflatoxins are a group of closely related toxic metabolites produced on feedstuffs by *Aspergillus flavus* (Ciegler and Lillehoy 1968) and *A. parasiticus* (Hesseltine 1970). Sargent *et al.* (1961) isolated aflatoxins from samples of highly toxic peanut meal by conventional extraction and concentration procedures. It has subsequently been found that the material originally isolated comprised several factors; the most common of which are aflatoxins B1, B2, G1 and G2. The distinguishing letters refer to the colour of the fluorescence exhibited on thin layer chromatograms during exposure to long wave ultraviolet light (B: blue, G: green). The suffixes refer to their respective positions on the chromatograms (Sargeant *et al.* 1963). Aflatoxin B1 is the most toxic of all the aflatoxins. Since the discovery of aflatoxins they have received intensive study because of their hepatotoxic and carcinogenic effects in domestic animals and man. Aflatoxin has been identified in a wide variety of foodstuffs includings maize, peanut, cotton-seed and palm kernels. Under natural conditions peanut constitute the most important contaminated commodity. Toxin production in peanut usually occurs subsequent to harvesting during either drying, processing or storage when the humidity is suitable for germination of *A. flavus* spores. Toxin production can also occur before harvest, particularly in corn and particularly where there has been insect damage to the crop. Not all strains of *A. flavus* are toxigenic and aflatoxin can also be produced by a variety of other fungi. The optimum conditions for growth and production of aflatoxin by A. flavus are a temperature of 30°C and relative humidity of 80-85 percent. The warm humid conditions of Indonesia favour the proliferation of aflatoxin producing fungi and it has been reported by Hetzel and Sutikno (1979) that high proportion of feedstuffs in Indonesia were contaminated with aflatoxins exceeding 50 ug/kg, i.e. to a higher degree than established as tolerable in many countries (Krough 1977). Thus for preventing aflatoxicosis it is necessary to ensure satisfactory harvesting, processing and storage methods which prevent the development of conditions suitable for the growth of *A. flavus* and aflatoxin production.

II. AFLATOXICOSIS IN ANIMALS

The study of this condition began in 1960 when thousand of turkey poults in the United Kingdom died from a didease which was initially termed "Turkey X disease" and which is now referred to as aflatoxicosis. The condition was acute and affected birds showed symptoms of inappetance, lethargy, a rapidly developing weakness, convulsions and death within 5-7 days of the onset of symptoms. Post mortem lesions consisted mainly of haemorrhage or necrosis of the liver and congested

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kidneys. Subsequent investigations have shown that a wide variety of animals are susceptible to aflatoxin, particularly turkey poults, duckings and rainbow trout. A few animals including horses, sheep and cats do not appear to be very susceptible. Some of the characteristic symptoms of the disease which have been reported in various animal species include jaundice in pigs and dogs, tenesmus and eversion of the rectum in calves and subcutaneous oedema in guinea pigs. Table 1 shown the various effects of aflatoxins in various animal species. In all animals, lesions occur typically in the liver which is the organ susceptible to the action of aflatoxin. Liver lesions include fatty infiltration, cirrhosis, haemorrhages and microscopically there is proliferation of bile-duct epithelial cells and fibrosis. In addition it is now known that aflatoxin is a higly potent hepato-carcinogen and the development of liver tumours has been studied in rats, ducks and rainbow trout after prolonged feeding of aflatoxins. Other significant aspects of chronic aflatoxicosis are failure to gain weight at a normal rate, reduced production and decreased resistance to infectious disease.

Table 1.
The acute and chronic effects of aflatoxins in animals (15)

Species	Stunting	BDP	VAC	Icterus	Depression	Death
Quail	+	+ +	+	+	+	+
Turkey	+	+ +	+ +	+	+	−
Ducklings	+	+ +	+ +	+ +	+	+
New Hempshire chicks	+	+ +	+ +	+	+	+
Broilers	+	+	+	+	+	−
Leghorns	−	+	+	−	−	−
Dogs	−	+ +	+ +	+	+	−
Pigs	+ +	+ +	+ +	+	+	+
Calves, Holstein	+ +	+ +	+ +	+	+	−

(Rough hair-coats, arched backs, severe straining oedema and haemorrhage)
BDP - Bile duct proliferation
VAC - Parenchymal cell vacuolation
- no effect; + moderate effect; + + severe effect

Among poultry, the duckling is the most sensitive species followed by the turkey poult, gosling, pheasant, chicken and quail (Allcroft 1969; Gumbman and Williams 1970; Muller et al. 1970). Although the chicken is considerably less susceptible to the effects of aflatoxins than are ducks and poults there is nevertheless quite a diversity of aflatoxins "susceptibility" between that various strains of chickens Abrams (1965) observed striking differences in succeptibility to aflatoxin in 17 different breeds and strains of pultry and game birds.

In addition to species and breed variation in susceptibility to aflatoxin there are another factors which influence the degree of response to these toxins. The factors are:

- age: young animals very susceptible
- sex: males more susceptible than femalse
- parasites: particularly those which effect the liver increase susceptibility
- method of ingestion: single large dose (acute vis a vis many small doses (chronic)
- nutritional status specially in respect of protein = deficiency increase the sensitivity of the liver to the effects of the aflatoxins
- vit K status: vit K will protect against decreased protrombim and reduce the lesion
- bile deficiency due to obstructive jaundice etc. will decrease absorption of vitamin K and A and predispose to greater hepatotoxicity

In Indonesia aflatoxicosis in ducks is the major problem in intensifying duck husbandry. Ginting (1983) reported that more than 50% of cases in ducks submitted to the diagnostic laboratories all over Indonesia were diagnosed as aflatoxicosis.

III. INTERACTION OF AFLATOXINS WITH OTHER DISEASE

The possibility of interactions between aflatoxicosis and other disease conditions has been considered for some time. Siller and Ostler (1961) described the isolation of salmonella from the internal organs of turkeys during field outbreaks of aflatoxicosis. Brown and Abrams (1965) consistently isolated salmonella from ducklings and chickens with typical aflatoxicosis. They also note a hypoproteinemia which included low levels of globulin and then suggested that aflatoxicosis induced a greater susceptibility to salmonella in avian species. Abrams (1965) hypothesized that susceptibility to other bacterial and viral disease would be affected similarly.

Smith *et at.* (1969) studied the relationship between aflatoxin and *Salmonella gallinarum* infections of chickens and concluded that both diseases exerted their effects on body weight and mortality independently and without interaction. The dose of *S. gallinarum* administered is not stated in this study but the level of aflatoxin (5 ppm of diet) was very high, and may have overshadowed any interaction. Wyatt and Hamilton (1975) however reported and interaction between a natural congenital infection of *Slamonella worthington* and aflatoxin on body weight. Further studies by Boonchuvit *et al.* (1975) have shown an interaction between four species of salmonella and aflatoxin. The effect of aflatoxin was manisfested by an increased mortality and increased frequency of isolation of salmonella from liver, a dramatic increase in anti-salmonella agglutinins and a decrease in total serum protein.

Hamilton and Harris (1971) demonstrated that aflatoxin ingestion increased the severity of *Candida albicans* infections in chickens. Severity of liver fluke *(Fasciola hepatica)* infection of calves is also increased by aflatoxin ingestion (Osuna *et al.* 1977). On the other hand the addition of aflatoxin to the ration of hamsters did not increase their susceptibility to *M. paratuberculosis* but rather seemed to decrease susceptibility to the bacillus (Larsen *et al.* 1975).

Edds *et al.* (1973) using New Hamsphire chickens showed that previous exposure to aflatoxin B1 (0.2 ppm) increased mortality and susceptibility to *Eimeria tenella* but did not interfere with the protection afforded by a coccidiostat (Amprolium). Wyatt *et al.* (1975) have confirmed this result with *E. tenella* infection, but in their study aflatoxin reduced the effectiveness of the coccidiostat (Monensin). MD vaccinated and non-vaccinated groups of chickens given aflatoxin B1 and subsequently exposed to caecal coccidiosis were more susceptible to challenge inoculation with MD virus than were similar groups of chickens not given aflatoxin (Edds *et al.* 1973). The bulk of the evidence therefore indicates an interaction between aflatoxin and suscepti bility to other diseases.This interaction appears to be manifested through and effect of aflatoxins on the immune system.

Effect of Aflatoxin on Immunity

Aflatoxin inhibits the development of acquired immunity and resistance to infection. Early observations on turkeys dead of aflatoxicosis in England in 1960 indicated that a large number of the turkeys had candidiases, a known opportunistic infection. Since that time, a number of investigations have shown that aflatoxin even at low concentration in poultry feed reduced resistance to infection with *Pasteurella multocida* (Pier and Heddleston 1970), *Salmonella* spp. (Smith *et al.* 1969), Marek's Disease Virus, Coccidia (Edds 1973) and *Candida albicans* Hamilton and Harris 1971).

The exact mechanism by which aflatoxin increased the susceptibility of young chicks to infectious agents is not understood. Giambrone *et al.* (1978a) indicated that aflatoxin had a marked effect on cell-mediated immunity in the chicken, as measured by the graft-versus-host and delayed hypersensitivity skin reactions. A decrease in cell-mediated immunity may explain why prior feeding of aflatoxin renders chicks more susceptible to caecal coccidiosis, a common intercellular parasitic disease of poultry. Since cell-mediated immunity has been shown to play a major role in the resistance to coccidiosis (Rose *et al.* 1975), a reduction in this immunologic function by aflatoxin could make chicks more susceptible to this disease. An effect of aflatoxins on cellular immunity is also indicated by others studies which ability of heterophils was inhibited in thickens receiving dietary aflatoxin (Chang *et al.* 1976).

Giambrone et al. (1987a) showed that aflatoxin caused a reduction in serum levels of Ig G and Ig A in chickens. Ig G is the major immunoglobulin in serum and is extremely important in neutralizing infectious agents. A reduction in Ig G production could result in an increased susceptibility of chicks to various common poultry pathogens. The great reduction in Ig A synthesis may provide and explanation as to why aflatoxin renders chicks more susceptible to *Candida albicans,* and enteric pathogen. Ig A is primarily responsible for production of local immunity, and a reduction in synthesis of Ig A could increase the susceptibility of chicks to local infections such as those in the gut. The impairment of Ig G and Ig A production could also be due to an inability of the thymus to switch over the production from Ig M to Ig G and Ig A (Bienestock *et al.* 1973). The concentration of Ig M was not altered by feeding aflatoxins, probably because the precursors for Ig M are produced during the later stages of embryonation (Cooper *et al.* 1972) Therefore, aflatoxin fed to newly hatched chicks would be too late to impair Ig M producing cells.

When aflatoxin B1 (0.25 to 0.5 ppm) was fed during the immunization period it impaired resistance to *Pasteurella multocida* in turkey poults and young chickens. On the other hand if aflatoxin consumption was discontinued prior to immunization then adequate immunity resulted and diminution of antibody response to *P. multocida* was not observed (Pier and Heddleston 1970). Pier *et al.* (1972) concluded that the impaired resistance to *P. multocida* infection in turkeys vaccinated against fowl cholera was not necessarily associated with antibody, as the deficit could be overcome by giving vaccinated birds either normal or immune serum prior to challenge inoculation.

Aflatoxin ingestion caused a reduction in complement activity in guinea pigs (thurston *et al.* 1972) and broiler chickens (Campbell *et al.* 1983), a delayed interferon formation in turkeys following Newcastle Disease Virus (NDV) inoculation (Pier *et al.* 1971) and impaired antibody response to Newcastle Disease vaccination in chickens (Boulton *et al.* 1982; Chenchev *et al.* 1978; Mohidin *et al.* 1981). All of these observation and further support to the suggestion that aflatoxins interfere with humoral immunity. However reduced resistance was not demonstrated with Newcastle Disease Virus (Pier *et al.* 1971) and on *Aspergillus fumigatus* infections (Pier *et al.* 1971) although aflatoxin decreases phagocytosis of *A. fumigatus* spores (Richard and Thurston 1975). Aflatoxin did not affect the antibody responce of guinea pigs to *Brucella abortus* antigen, although complement activity was depressed as was alpha, beta globulin and total serum protein concomitant with elevated levels of gamma-globulin (Thaxton *et al.* 1974). Giambrone *et al.* 1978b) observed a significantly high mortality and severely depressed body weights of young chickens fed 2.5 ug of aflatoxin per g of diet from hatching until 4 weeks old and infected with Infectious Bursal Disease Virus (IBDV). IBDV infection at 1 day of age dramatically suppressed humoral immunity whereas dietary aflatoxin is incriminated in the impairment of cell-mediated immunity.

It was speculated that the combined effect of IBDV and Aflatoxin could, result in a severe depression in the immunological responsiveness of young birds, rendering them highly susceptibility to many common organisms of usually low pathogenicity.

Aflatoxin suppresses antibody formation in mice

given typhoid vaccine (Galikeev *et al.* 1968). In chickens, aflatoxin consumption has been shown to result in a lag in production of hemaglutinins to sheep RBC (S-RBC) (Thaxton *et al.* 1974) but not significant effect on the production of natural agglutinins to rabbit red blood cells (R-RBC) (Giambrone *et al.* 1978a). These results may be explained by the fact that S-RBC are a thymic (T) dependent antigen. Since T dependent antigens require both T and bursal (B) cells for antibody synthesis, dietary aflatoxin could directly impair antibody formation to S-RBC by altering T cell helper function. In contrast, T cells are not needed for the production of natural agglutinins to R-RBC (Toivanen *et al.* 1972).

Therefore the lack of a significant effect of dietary aflatoxin on the production of natural agglutinins to R-RBC can be explained by the failure of aflatoxin to significantly alter B-cell function. A study by Giambrone *et al.* 1982), however, failed to demonstrate the effect of aflatoxin B1 on humoral and cell mediated immune responses in broilers.

The bursa of Fabricious and the Thymus are both important components of the avian immunological system (Cooper *et al.* 1965; Glick 1970) and they were reduced in size by 30% and 55% respectively, when chicken ate a diet containing 10 ug of aflatoxin per gram of feed (Thaxton *et al.* 1974). This phenomena may also account at least in part for aflatoxin induced three additional areas in which aflatoxins could exert an immunosuppressive effect; the mechanism for the aflatoxin induced immunosuppressive is not well defined but could be associated with three biochemical processes operating either independently or together eg.:

a. Immunological Synthesis

Aflatoxins have been demonstrated to inhibit RNA polymerase *in vivo* and subsequently to limit protein synthesis (Lafarge and Frayssinet 1970). Immunosuppression by aflatoxin then could be the result of inhibition of the synthesis of specific immunoglobulins.

b. Immunoglobulin Hydrolysis

Aflatoxins can cause rapid and dramatic increases in the specific activity of lysosomal enzymes in skeletal muscle and liver of chickens (Tung *et al.* 1971) and a dose related decrease in tissue strength and integrity (Tung *et al.* 1970). Since lysosomes and their hydrolytic enzymes are involved in the extracellular and intracellular digestion of macromolecules (De Duve and Wattiaux 1976) Aflatoxin could be an immunosuppressant by virtue of its ability to stimulate lysosomal degradation of immunoglobulins.

c. Impaired Antigenic Responce

Aflatoxin inhibits the reticuloendothelial system in a dose-related fashion (Micheal *et al.* 1973). The system is responsible for the removal of foreign particulate matter from the circulation and for the protection of the tissues from invasion by noxious organisms.

CONCLUSION

The effects of aflatoxin on immunity and resistance to microbial invasion seem to vary according to the animal species and to the agents involved. The effects seem to be related in part to depression of non-humoral substances such as complement, interferon and in part to altered interaction between immunogen and aflatoxin influenced host tissues. In some instances the effect may be related to depressed antibody formation or cell-mediated immunity. Regardless of the mechanism, the end result of impaired resistance and immunologic response is one of real economic importance to the livestock industry of Indonesia.

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