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 infectious 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.

Aflatoxin is a racemic racemic mixture of four isomers known as aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2. 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 (Sargent et al. 1963). Aflatoxin B_1 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 including maize, peanut, cotton-seed and palm kernels. Under natural conditions peanut constitute the most important contaminated commodity. Toxic 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. Toxic 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 Hezel and Sumino (1979) that high proportion of feedstuffs in Indonesia were contaminated with aflatoxin exceeding 50 μg/kg, i.e. to a higher degree than established as tolerable in many countries (Krouth 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.

I. INTRODUCTION

Aflatoxins are a group of closely related toxic metabolites produced on foodstuffs by Aspergillus flavus (Clotet and Liddleby 1968) and A. parasiticus (Hoseltine 1970). Sargent et al. (1961) isolated aflatoxins from samples of highly toxic peanut meal by conventional extraction and concentration procedure. It has subsequently been found that the material originally isolated comprised several factors, the most common of which are aflatoxins B_1, B_2, G_1 and G_2.

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kidneys. Subsequent investigations have shown that a wide variety of animals are susceptible to aflatoxin, particularly turkey pouls, ducks, 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 shows the various effects of aflatoxin 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 histologically there is proliferation of bile-duct epithelial cells and cirrhosis. In addition it is now known that aflatoxin is a highly potent hepatocarcinogen 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 aflatoxin in animals (15) |
|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Species             | Symptoms             | BDP                | VAC                | Auran              | Depression          | Death              |
| Quail               | ++ ++ ++ ++ ++      | +                  | +                  | +                  | +                   | +                  |
| Turkey              | ++ ++ ++ ++ ++      | +                  | +                  | +                  | +                   | +                  |
| Ducks               | ++ ++ ++ ++ ++      | +                  | +                  | +                  | +                   | +                  |
| New World pigeons   | ++ ++ ++ ++ ++      | +                  | +                  | +                  | +                   | +                  |
| Pigeons             | ++ ++ ++ ++ ++      | +                  | +                  | +                  | +                   | +                  |
| Geese               | ++ ++ ++ ++ ++      | +                  | +                  | +                  | +                   | +                  |
| Cocks               | ++ ++ ++ ++ ++      | +                  | +                  | +                  | +                   | +                  |
| (Rough-hair-coats, dirtied backs, severe subcutaneous oedema and haemorrhage) |
| BDP - Bile duct proliferation |
| VAC - Parasitoidal cell vacuolation |
| Auran - Auran reduction |
| Depression - Depression increase |
| Death - Death due to aflatoxicosis |

Among poultry, the duckling is the most sensitive species followed by the turkey poult, goose, pheasant, chicken and quail (Adcroft 1969; Dziuban and Williams 1970; Mulier et al. 1970). Although the chicken is considerably less susceptible to the effects of aflatoxins than are ducks and pouls there is nevertheless quite a diversity of aflatoxin 'susceptibility' between that various strains of chickens. Abrams (1965) observed striking differences in susceptibility to aflatoxin in 17 different breeds and strains of poultry and game birds. In addition to species and strain 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 females
- parasites: particularly those which affect the liver increase susceptibility
- method of ingestion: single large dose (acute) vs a vs many small doses (chronic)
- nutritional status especially in respect of protein deficiency increase the sensitivity of the liver to the effects of the aflatoxins
- liver K and Na content: resistance against decreased protein intake and reduce the lesion
- bile deficiency due to obstructor 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 DISEASES

The possibility of interactions between aflatoxins and other disease conditions has been considered for some time. Sitter and Oster (1964) described the isolation of salmonella from the internal organs of turkeys during field outbreaks of aflatoxicosis. Brown and Abraham (1965) consistently isolated salmonella from ducklings and chickens with typical aflatoxins. They also note a hypoproteinaemia which included low levels of globulin and then suggested that aflatoxins induced a greater susceptibility to salmonella in avian specims. Abrams (1965) hypothesized that susceptibility to other bacterial and viral disease would be affected similarly.

Smith et al. (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 (1978) however reported that interaction between a natural congenital infection of Salmonella typhimurium and aflatoxin on body weight. Further studies by Booschovitch et al. (1975) have shown an interaction between fowl species of salmonella and aflatoxin. The effect of aflatoxin was manifested by an increased mortality and increased frequency of isolation of salmonella from liver, a dramatic increase in anti-salmonella antibodies and a decrease in total serum protein.

Hamilton and Harris (1973) 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 (Onuma et al. 1977). On the other hand the addition of aflatoxin to the ration of hamsters did not increase their susceptibility to M. parastrabeculosis but rather seemed to decrease susceptibility to the bacillus (Laren et al. 1975).
Edds et al. (1973) using New Hampshire chickens showed that previous exposure to aflatoxin B1 (0.2 ppm) increased mortality and susceptibility to Escheria coli but did not interfere with the protection afforded by a coccidistat (Ampromilum). Wyatt et al. (1975) have confirmed this result with E. coli, but in their study aflatoxin reduced the effectiveness of the coccidistat (Montecum). MD vaccinated and non-vaccinated groups of chickens given aflatoxin B1 and subsequently exposed to coccidia coccidies 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 susceptibility 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 turkey dead of aflatoxicosis in England in 1960 indicated that a large number of the hens had candidiasis, a known opportunistic infection. Since that time, a number of investigations have shown that aflatoxin even at low concentrations in poultry feed reduced resistance to infection with Pasteurella multocida (Pier and Hedelton 1970), Salmonella spp. (Smith et al. 1949), Marek's Disease Virus, Coccidia (Edds 1973) and Candida albicans (Crum and Harrison 1971). The exact mechanism by which aflatoxin increased the susceptibility of young chicks to infectious agents is not understood. Giambonne 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 coccidial coccidies, a common intercellular parasitic disease of poultry. Since cell-mediated immunity has been shown to play a major role in the resistance to coccidies (Rose et al. 1975), a reduction in this immunologic function by aflatoxin could make chicks more susceptible to this disease. An effect of aflatoxin on cellular immunity is also indicated by other studies which ability of heterophil cells was inhibited in chicks receiving dietary aflatoxin (Crum and Johnson 1976).

Giambonne et al. (1978a) showed that aflatoxin caused a reduction in serum levels of IgG and IgA in chickens. IgG is the major immunoglobulin in serum and is extremely important in neutralizing infectious agents. A reduction in IgG production could result in an increased susceptibility of chicks to various common poultry pathogens. The great reduction in IgA synthesis may provide an explanation as to why aflatoxin renders chicks more susceptible to Candida albicans and enteric pathogens. 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 IgG and IgA production could also be due to the ability of the aflatoxin to switch over the production from Ig M to Ig G and Ig A (Bienesek et al. 1973). The concentration of Ig M was not altered by feeding aflatoxin, but probably inhibition of the precursors for Ig A are produced during the later stages of embryogenesis (Cooper et al. 1972). Therefore, aflatoxin fed to newly hatched chicks would be too late to impair Ig A production.

When aflatoxin B1 (0.25 to 0.5 ppm) was fed during the immunization period it impaired resistance to Pasteurella multocida in turkey poultls 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 Hedelton 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. 1971) and broiler chicks (Campbell et al. 1983), a delayed interferon formation in turkey following Newcastle Disease Virus (NDV) inoculation (Pier et al. 1971) and impaired antibody response to Newcastle Disease vaccination in chickens (Boulot et al. 1982; Chenevet et al. 1978; Mahdavi et al. 1981). All of these observation and further support to the suggestion that aflatoxin interferes 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 response of guinea pigs to Brucella abortus antigen, although complement activity was depressed as was alpha, beta globulin and total serum protein content with elevated levels of gamma globulin (Thurston et al. 1974). Giambonne et al. (1978b) observed a significantly high mortality and severely depressed body weights of young chickens fed 2.5 mg of aflatoxin per lb of diet starting from 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 susceptible to many common organisms of usually low pathogenicity.

Aflatoxin suppresses antibody formation in mice.
given typhoid vaccine (Galikiev et al., 1968), in chickens, aflatoxin consumption has been shown to result in a lag in production of hemagglutinins to sheep RBC (S-RBC) (Thaxton et al., 1974) with no 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. (1968), however, failed to demonstrate the effect of aflatoxin B1 on hemagglutinin and cell mediated immune responses in broilers.

The burst of Fabriciour 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 µg 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 Fraynstein, 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 macrophages (De Duve and Wattiaux, 1976) Aflatoxin could be an

c. Impaired Antigenic Response

Aflatoxin inhibits the reticuloendothelial system in a dose-related fashion (Michel & al., 1979). 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.

immunosuppression by virtue of its ability to stimulate lysosomal degradation of immunoglobulins.

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 of cell-mediated immunity. Regardless of the mechanism, the end result of impaired resistance and immunologic response is one of real economic importance to the live stock industry of Indonesia.

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