Immuno-modulatory and Growth-inhibitory Effects of Mycotoxins (Aflatoxin B1) in Broiler Chickens and the Reversal Influence of Hydrated Sodium Calcium Aluminosilicate

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Abstract
Eighty, day-old broiler chicks were primed with live ND and IBD vaccines at 7 and 9 days of age and boosted at the age of 21 and 28 days, respectively. At 3 weeks of age, chicks were divided into 5 equal groups A, B, C, D and E. Groups A, B and C were fed on aflatoxin contaminated feed and 1, 2 and 3 g of Hydrated Sodium Calcium Alumino Silicate (HSCAS)/kg of feed respectively. Group D was raised on contaminated feed without binder (positive control) and group E on normal feed without binder (negative control) for a period of four weeks. Maximum body weight gain was observed in group E and minimum in group D. There was a dose related increase in the weight gains of groups A, B and C. The minimum HI titres against NDV were found in group D and maximum in group E. The gradually increasing dosage of the binder in the feed of groups A, B and C manifested a dose related increase in HI antibody titres. Maximum IHA titres against IBD were found in group E and minimum in group D. Groups A, B and C also manifested a dose related increase in IHA titres against IBD. The mortality recorded in groups A, B, C, D and E was 31.25, 18.75, 18.75, 43.75 and 0.0 percent, respectively. Mycotoxin-binder at the rate of 3 g/kg of feed gave better results in terms of body weight gain, humoral immune response and better protection against aflatoxicosis compared to the dose rates of 1 and 2 g/kg of feed.

Introduction
Aflatoxins, a group of closely related, biologically active mycotoxins, are produced by strains of Aspergillus flavus and A. parasiticus (Mishra and Daradhiyar, 1991). Of the four most familiar aflatoxins B1, B2, G1 and G2, are the most common and most toxic (Ketterer et al., 1982). These aflatoxins have been shown to cause immunosuppression (Boulton et al., 1982; Ilgaz, 1985; Rizvi and Shakoori, 1992; Tuekam et al., 1992, Mohiuddin, 1993), growth retardation (Ghosh et al., 1990; Weibking et al., 1992) and high mortality (Sabri et al., 1989; Siddique and Javed, 1989). The post-mortem lesions include enlarged, pale yellow, friable livers with pin point haemorrhage; swollen, pale white kidneys with prominent tubules and petechial haemorrhages, varying degree of enteritis, hydropneumonicardium with cyanosis of myocardium (Murthy et al., 1986; Balachandran and Ramakrishnan, 1987; Arshad et al., 1992).

Different workers (Huff et al., 1992; Kubena et al., 1992; Abo-Norag et al., 1995) have used mycotoxin binder called hydrated sodium calcium aluminosilicate (HSCAS) as feed additive to minimise the adverse effects of mycotoxins present in feed.

Pakistan, because of its high humidity, high temperature and inadequate storage facilities is an ideal country for the problems of aflatoxins. Aflatoxins have been isolated from different feed-stuffs used mainly for preparation of poultry feed. These include corn, corn by-products, cotton-seed meal/cake, broken rice, sunflower meal/cake, sorghum and rapeseed meal/cake (Afzal et al., 1979; Chaudhary and Afzal, 1982).

Keeping in view the immunosuppressive effects of aflatoxins and the reported beneficial effects of HSCAS, the present study was conducted to determine the effect of aflatoxins on growth and humoral immune response of broiler chickens following ND and IBD vaccinations and the efficacy of HSCAS to check the adverse effects of aflatoxins on broiler chickens.

Materials and Methods
A total of 80, day-old broiler chicks were fed on normal poultry feed up to three weeks of age. AK chicks were primed with live ND (BioSota, Bioteke) and IBD (IBA-VAC, D-78 strain, Neuva) vaccines at the age of 7 and 9 days, respectively. The birds were boosted with live ND (Mukteswar strain, VR1) and IBD (1BA-VAC, D-78 strain Neuva) vaccines at the age 21 and 28 days, respectively. At three weeks of age, the chicks were divided into five group A, B, C, D and E of 16 chicks each. Five chicks from each group were randomly picked up and weighed at the start of the experiment. Thus average body weight for each group was calculated. Groups A, B and C were fed on aflatoxin contaminated feeds containing 1, 2 and 3 gm of hydrated sodium calcium alumina silicate/kg of feed, respectively for four weeks. Group D was given contaminated feed without binder to serve as positive control and group E was given normal feed without binder to serve as negative control. The presence of aflatoxins and its level was determined by thin layer chromatography (TLC) method described by Jones (1972). Serum samples were collected at weekly intervals starting from the first week of
feeding the test feed till the expiry of the experiment. The serum antibody titers against ND and IBD were determined through HI and IHA tests, respectively. Average weight gain were also determined at the accomplishment of the experiment for each of 5 experimental groups by averaging five random observations taken. Due records of mortality were also maintained through daily examination of all the experimental chickens. An dead birds found in each group were picked up and autopsied to record gross pathological lesions in various organs. The data about HI and IHA titers was analysed statistically through completely randomised design (CRD) and Duncan’s multiple range (DMR) test using MSTAT-C computer package.

**Results**

Analysis of experimentally contaminated feed by TLC method revealed that aflatoxin B1 was present in the feed at the levels of 100-500 $\mu$g/kg of the feed. As regards HI titres against ND, maximum geomean titer (GMT) was found in negative control group E while minimum GMT was found in positive control group D (Fig. 1). The gradually increasing doses of the binder in feed of groups A, B and C manifested a dose related response in geomean HI antibody titres. A statistical analysis of the data showed that differences in HI titers in all groups were non significant at first week post-feeding and highly significant ($P<0.01$) thereafter throughout the experiment.

For IHA titres against IBD, Bur-706 and D-78 strains were used as antigens. Regarding IHA titres against IBD using Bur-706 strain, the maximum GMT was found in group E and the minimum GMT in group D. The gradually increasing doses of toxin binder in the feed of groups A, B and C manifested a dose related response in IHA titers (Fig. 1). Statistical analysis showed that mutual differences in humoral immune response of experimental groups were non significant at first and second weeks and highly significant ($P <0.01$) at third and fourth week post-feeding.

Using D-78 strain as antigen, the general trend of humoral immune response remained almost the same, however, relatively lower IHA titers were observed compared with Bur-706 strain. Thus maximum GMT was found in group E and minimum in group D (Fig. 1). The gradually increasing doses of the binder in the feed of groups A, B and C manifested a dose related response in HA titers. The GMT differences were highly significant ($P <0.01$) at weeks third and fourth and non-significant at weeks first and second.

The average body weights recorded at the start of the trial were 658, 657, 658, 660 and 660 gms for group A, B, C, D and E, respectively. The average body weights for groups A, B, C, D and E calculated at the accomplishment of the trial (7 weeks) were 1148, 1335, 1444, 1030 and 1590 gm, respectively. Thus net weight gains for groups A, B, C, D and E were recorded as 490, 678, 786, 370 and 930 gm respectively.

The mortality recorded in groups A, B, C, D and E was 31.25, 18.75, 18.75, 43.75 and 0.0 percent, respectively. The post-mortem lesions included enlarged, pale yellow and fragile livers with pinpoint haemorrhages. Kidneys were swollen, pale white with prominent tubules and haemorrhages. Heart showed hydropericardium, pinpoint haemorrhages and cyanotic appearance of myocardium. Intestines revealed haemorrhagic enteritis. Spleen was also enlarged with mottled appearance in some cases.

**Discussion**

Mycotoxicosis, in the context of its significance, has always been a subject of prime interest. In hot humid weather of the tropics, mycotic growths are very common to appear on any suitable nutrient substrate. During the phase of active mycotic growth, not only reproductive cells would be formed but the potent extra cellular proteinous metabolites named generally as mycotoxins are also produced. The cytological damage induced by the ingestion of such detrimental products varies owning to a number of factors such as type and amount of mycotoxin ingested, species susceptibility, age, period of exposure stress induced by concurrent infections, malnutrition and weather harshness, etc.

Reviewing in the context of relevant literature available, has been reported that the acquired immunity against ND broiler chickens is acutely suppressed if previously fed aflatoxin harbouring feed (Ilgaz, 1985). Tuekam et al. (1992) used four dietary concentrations (0, 250, 500 and 700 ppb) of AFBI and reported that antibody titres against infectious bronchitis virus vaccine decreased ($P<0.05$) at all three concentrations of AFBI. In another study, ND HD titres had enormously decreased in hens vaccinated against ND and fed on 500 $\mu$g/kg crude AF (Boulton et al., 1982).
Rizvi and Shakoori (1992) demonstrated that the immunological response was extremely poor (comparable with those of unvaccinated control group) in broiler and layer chicks vaccinated against ND. The vaccinated toxin-free control birds were reported to develop significantly higher (P<0.05) HI titres. In agreement with the conclusions drawn by Mohiuddin (1993), it may be suggested that all vaccination failures against diseases predominantly ascribe to the immunosuppressive effects of aflatoxins in feeds, eventually leading to high mortality, high morbidity and heavy economic losses.

The results of the present study are fully supported by the findings of Siddique and Javed (1989) who noted 12-47 percent mortality in broilers fed on diet containing 22-96 ppb mycotoxins. The mortality rate in the present study was also proportional to the level of mycotoxin present in the feed.

The post-mortem lesions observed during the present study are in line with those reported by Murthy et al. (1986), Balachandran and Ramakrishnan (1987) and Arshad et al. (1992).

Regarding the growth inhibitory effects of aflatoxins, the results of the present study coincides with those of Ghosh et al. (1990) who also observed a poor growth rate in broilers fed on diet containing 30-50 ppb aflatoxins. Weibking et al. (1992) also reported a reduction in body weight gains of turkey pouls fed on 200 ppb aflatoxins. Regarding the beneficial effects of HSCAS, the results of the present study are fully supported by those of Huff et al. (1992), Kubena et al. (1992) and Abo-Norag et al. (1995) who used 0.5 per cent HSCAS as feed additive to diminish the growth inhibitory effects of 3.5 mg AF/kg feed and reported that HSCAS reduced the toxic effects of AF on body weights of chickens.

References