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Research Article

EFFECT OF OCHRATOXIN-A FEEDING ON PROTECTION IN BROILER CHICKS FOLLOWING VACCINATION AND CHALLENGE WITH SALMONELLA GALLINARUM

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ABSTRACT

Effect of ochratoxin-A feeding on protection in broiler chicks was studied following vaccination and challenge with *Salmonella enterica* serotype Gallinarum (*Salmonella* Gallinarum).There were 25% shedders two weeks post challenge in OA fed group B as compared to 11.76% in vaccinated group A and 50% in control group C respectively. Mean $\log_{10}$ cfu g^{-1} of faeces in OA fed broilers was 3.41 as compared to 5.17 in control group C and 2.39 in vaccinated and without OA fed group B . Following challenge, 54.55% birds survived in vaccinated and OA fed group B as compared to 27.27% in control group C and 77.27% in vaccinated without OA fed groupB. The per cent protective index of adjuvanted *Salmonella* Gallinarum vaccinal preparation in chicks of group A was found to be 59.09, 77.27, 68.18 and 22.73 respectively, based on bacteriological examination, gross pathological lesions, histopathological lesions and mortality patterns. It was much lower in vaccinated, OA fed group B i.e. 31.81, 45.45, 40.90 and 45.45 respectively and in control group C, it was 9.09,31.81,18.18 and 72.73, respectively. Based on different protection parameters OA fed group B showed considerably lower protection as compared to vaccinated and without OA fed group A chickens.

Key words: Ochratoxin- A, *Salmonella* Gallinarum, broiler chicks

The most common and toxic ochratoxins is ochratoxin-A (OA). It has been found as a natural contaminant of feed stuffs in many countries. Ochratoxin-A has been reported in corn, barley (Fischback and Rodricks, 1973), oats, mixed feeds, dried white beans and peanuts including dry fish, black pepper and rice. (Prior, 1976) Ochratoxin-A is primarily thought to be a nephrotoxin (Huff *et al.*, 1975) with some secondary hepatotoxicity. The effect of ochratoxin-A in chickens is reflected in poor growth rate, decreased egg production and lowered feed conversion ratio. As an immunosuppressive toxin, OA might be expected to predispose the birds to several secondary infections like *E.coli, Salmonella typhimurium* infection, New Castle disease and inclusion body hepatitis etc. (Sandhu *et al.*, 1998, Sakthivelan and George, 2002).The information about immunosuppressive effect of ochration-A in relation to immunoprophylaxis against *S.* Gallinarum in broilers is scanty. The effect of ochratoxin-A feeding on immunoprotective efficacy of killed *Salmonella* Gallinarum vaccine in broiler chicken observed are described in present communication.

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MATERIALS AND METHODS

Vaccine preparation: The stock culture of *S.* Gallinarum was grown in brain heart infusion agar in Roux flasks at 37°C for 24h and harvested by centrifugation at 2000x g for 30 min. normal saline solution. The organisms were suspended in (NSS) to make an approximate concentration of 2×10^{10} cfu 0.2 ml^{-1}. The culture was inactivated with 0.5% formalin 20-25°C for 24 h. The cell suspension, was mixed with equal volume of sterile aluminium hydroxide gel adjuvant. The final vaccine prepared contained approximately, 2×10^{10} cfu $(0.4 \text{ ml})^{-1}$.

Immunization and feeding schedule: Broiler chicks were randomly divided into three treatment groups A, B and C consisting of forty six chicks in each. Chicks of group A and C were given balanced and unmedicated broiler

mash. *Aspergillus ochraceus* culture containing 80.0 ppm ochratoxin-A was used to induce ochratoxicosis in broiler chicks. Chicks of group B were given ochratoxin-A @ 2.0 ppm in broiler mash from day one. Group-A Chicks were immunized subcutaneously(s/c) with aluminium hydroxide gel adjuvanted whole cell formalin-killed vaccinal preparation of *S.* Gallinarum(SG-FKV) Group-B Chicks were fed with ochratoxin-A and were immunized similar to group A chicks(SG-FKV-OA).Group-C chicks served as a control (AC)and were mock-immunized s/c with 0.4 ml sterile aluminium hydroxide gel adjuvant.

Challenge of birds: The chicks of all the three groups were challenged s/c with $4x10^{10}$ cfu 0.5 ml^{-1} (4 LD_{50}) of *S.* Gallinarum s/c at four weeks post-immunization (Rana, 1997). The challenged chicks of each group were monitored regularly upto two weeks for specific clinical signs, mortality pattern, excretion pattern of challenge organisms, quantitation of challenge organisms in faeces. From dead chicken, bacteriological examination, gross and histopathological examination was done from vital organs to study the efficacy of the vaccine. Per cent protection afforded by the vaccine was expressed as:

$$\% \text{ protection} = \frac{\text{Total number of chickens without isolation/lesions}}{\text{Total number of chickens examined}} \times 100$$

Statistical analysis: Data generated during the experiment was analysed statistically to draw inferences using completely randomized design (CRD).

RESULTS AND DISCUSSION

Excretion pattern of the challenge organisms was undertaken to assess the immunoprotective efficacy of the vaccine. The proportion of chickens shedding the challenge organism was considerably high during first week post challenge (PC) in all the groups and thereafter considerable declining trend in the proportion of shedders was observed. The number of shedders at two weeks PC, were found to be 11.76%, 25% and 50% in chicks of groups A, B, and C, respectively (Fig.1). The clearance of the challenge organisms has been used as one of

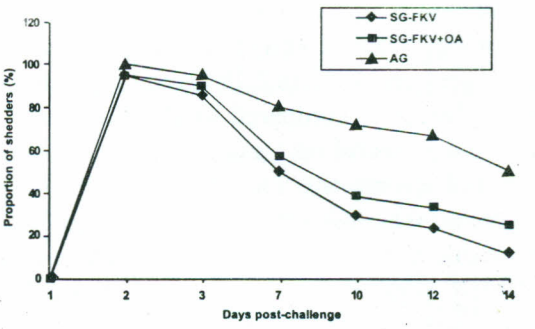

Fig 1. Excretion pattern of the challenge organisms in immunized chickens surviving challenge with *S.* Gallinarum.

the criteria to evaluate the potency of any immunizing agent (Davies and Kotlarski, 1974). The clearance of the challenge organisms indicates the absence of a carrier state. *Salmonella* infection may cause immunosuppression depending upon the type of strain, thereby facilitating the establishment of a carrier state and enhancing the frequency of secondary infections (Curtiss *et al.*, 1993). Blanden *et al.* (1966) stated that the rate of clearance of the challenge organisms is dependent on the cellular immunity rather than the humoral immunity. The higher proportion of shedders and slow rate of clearance of challenge organisms noticed in toxin fed group B as compared to group A in the present study can be due to the impairment of cellular immune response in these birds by ochratoxin-A.

Quantitation of challenge organisms using cloacal swabs was undertaken to study the multiplication and persistence pattern of challenge organisms in various groups of chickens (Fig 2).

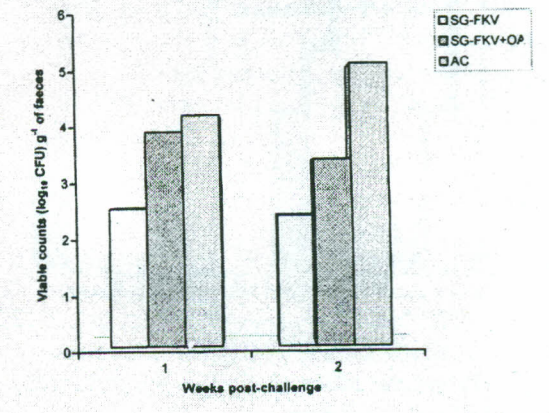

Fig 2. Quantitation of challenge organisms in faecal samples of immunized chickens following challenge with *S.* Gallinarum.

There was a significant ($P<0.05$) reduction in mean $\log_{10}$ viable count of challenge organisms in group A chicks with 2.39 mean Log_{10} cfu per g^{-1} of faeces as compared to OA fed group B with 3.41 mean Log_{10} cfu per g^{-1} of faeces. In OA fed group the clearance of challenge organism was less effective and it might have been due to immunosuppression. Fukata *et al.* (1996) reported that the number of *S.* Typhimurium in both the duodenal and caecal contents of chickens given at high dose of OA increased significantly when compared with the control birds. They also suggested that OA was one of the factor that affect the susceptibility of chickens to *Salmonella* colonisation.

Spleen was the most common organ that was found positive for isolation of challenge organism followed by liver and bile. The per cent protection index based on bacteriological examination from vital organs was found to be highest in group A with a protective index of 59.09%, as compared to 31.81% in OA fed group B and 9.09% in control group C (Fig 3). Similar findings of *S.* Gallinarum isolations in broilers fed with aflatoxins were also reported by Rana, 1997.

Michael *et al.* (1973) demonstrated reduction in isolation of *Salmonella* from liver of chicks due to impairment reticuloendothelial system by aflatoxicosis. Chang and Hamilton (1980) observed decreased phagocytic activity of heterophils in broiler due to ochratoxicosis that could also account for the increased isolation of *Salmonella* organisms from the liver as observed in the present study.

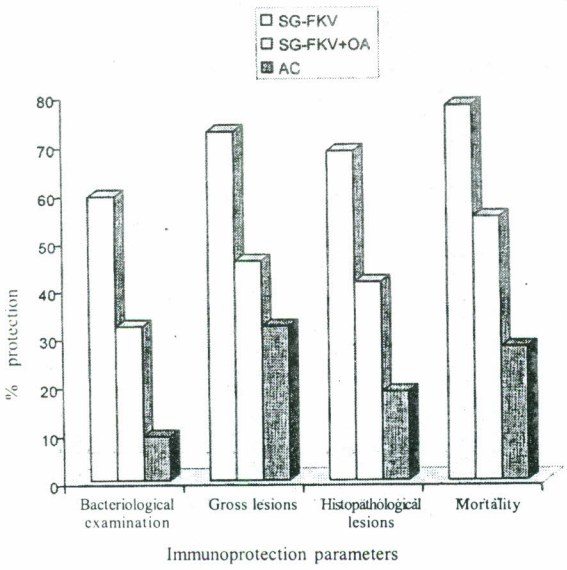

Fig 3. Protective efficacy of adjuvanted formalin-killed vaccine preparation of *S.* Gallinarum in chickens based on different immunoprotection parameters.

The gross pathological changes observed in group B chickens fed with OA were more severe than other groups. The lesions observed were hepatomegaly, distended gall bladder , pale and enlarged kidneys, visceral gout and atrophy of bursa of Fabricius and thymus, besides generalized congestion and haemorrhages. The enlargement of the kidneys in the OA fed group is in conformity with the findings of Manning and Wyatt (1984)and Hegazy and Adachi(2000). Similar other gross pathological lesions were also observed by Dwivedi and Burns (1984) and Sakthivelan and George (2002). Based on gross pathological lesions a protection index of 77.27%, 45.45% and 31.81% per cent was observed in the chickens of group A, B and C, respectively (Fig 3). Rana (1997) observed 72.41% protection in chicks vaccinated with SG-FKV, subcutaneously.

Histopathological lesions observed in kidneys of OA fed chickens of, group B were more severe as compared to other groups. The lesions observed in kidneys were tubular degeneration with congestion, haemorrhage and mononuclear cell infilteration. Mohiuddin (1992) and Sakthivelan and George (2002) observed dilatation of the tubules with occasional hyaline casts and intertubular haemorrhage. The observed pathological changes revealed that OA is a potent nephrotoxin in young broiler chicken in contrast to aflatoxin which is considered to be more hepatotoxic. Marked depletion of lymphocytes from spleen and bursa of Fabricius was observed in group B chickens. The lymphocyte depletion and immunosuppression during the infection may play a critical role in the development of *Salmonella* carrier states in chicks (Hassan and Curtiss, 1994).On histopathological basis, protection index of 68.18%, 40.90% and 18.18% was observed in chickens of group A, B and C, respectively. Batra (1993) reported 48.57 per cent protection levels afforded by the *S.* Gallinarum whole-cell

formalin-killed vaccine in aflatoxin fed chicks based on histopathological lesions. No such reports of protection indices on histopathological observation following feeding of ochratoxin-A are currently available.

The per cent protection based on mortality pattern was found to be 77.27%, 54.55% and 27.27% in chicks of group A, B and C respectively (Fig 3). Batra (1993) and Rana (1997) while studying the pathogenicity of killed *S.* Gallinarum vaccinal preparation in aflatoxin fed chicks observed the similar pattern.

The relative roles of humoral versus cell-mediated immunity in the resistance of chickens to infection with *Salmonella* species has not been well established. However, evidences suggest that it is the cell mediated immune response which is more important than the humoral immune response in protection since high level of circulating antibodies may be detected before any reduction in the numbers of *Salmonellae* is recorded (Lee *et al.*, 1981). Lax *et al.* (1995) observed both humoral and cell mediated immune responses in acquiring the resistance to *Salmonella* infection. In the present study, the adjuvanted whole-cell formalin-killed vaccinal preparation of *S.* Gallinarum revealed a good level of immunoprotection in the chickens of group A. The vaccine however provided comparatively much lower level of protection in the group B chicken which were suffering from ochratoxicosis .

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