EFFICACY OF DIFFERENT IMMUNOMODULATORS IN AFLOTOXIN INDUCED IMMUNOSUPPRESSED CHICKEN

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Abstract: Aflatoxicosis is one of the major cause of immunosuppression in poultry. The present study was carried out in growing broiler chicks divided in to six groups of sixteen each. Chicks of groups I to IV received 1 ppmaflatoxinper day for 2 weeks and chicks of group V served as healthy vaccinated control. From 3rd to 6th week, chicks received tuftsin (10 ug/bird), stresroak (10ml/100 birds in water) and zinc sulphate (40 ppm/bird in water) as immunomodulator for groups I, II and III, respectively. On 7th day pre vaccinated titres against NDV (T-dependent) and *S.gallinarum* (T-independent) for all groups were estimated by micro HI and SAT, respectively.From 3rd to 5th week, group I showed significantly(P<0.01) higher HI titres than those of group II, III and IV. SAT titres with *S.gallinarum* antigen also showed similar results. At the termination of the experiment the order of seroconversion to T-dependent antigen was I>V>II,III>IV, while the seroconversion to T-independent antigen was I>V>II>III>IV.Thetuftsin treatedbirds showed improved body weight compared to other immunomodulators. The results indicate thattuftsin proved superior as immunomodulator followed by sresroak and zinc sulphate in restoration of immune response in aflatoxin induced immunosuppression.

Key words: aflatoxin, immunomodulators, poultry.

Introduction: Aflatoxins are natural contaminants of poultry feeds and feed ingredients and cause liver damage, immunosuppression,reduced growth rate and increased mortality in broilers (UmayaSuganthi et al., 2011). The aflatoxin also causes vaccination failures and heavy mortality due to infection with a wide spectrum of infectious microorganisms (Gabal and Azzam, 1998) resulting huge economic loss to the poultry farmers. Among the aflatoxins, AFB1 was identified to be the most toxic and most prevalent compound (Murphy et al., 2006).

Since there is dearth of information, the present study was under taken to know the efficacy of three different immunomodulators namely tuftsin (Sigma, USA),stresroak (Herbal product from M/S DaburAyurved Limited, India) and zinc sulphate (Qualigens) in restoration of normal immunity in experimentally induced immunosuppression due to aflatoxin in chicken. Further, the seroconversion to T- dependent and T-independent antigens, average body weight gains and challenge tests were performed to find out the role of these immunomodulators in immunosuppressed chicks.

Materials and Methods: AspargillusParasiticus NRRL 2999 culture, maintained at Project Directorate on Poultry (ICAR), Rajendranagar, Hyderabad was used to produce aflatoxinon rice by the method of Shot well et al. (1966). The aflatoxin extracted from dried rice culture was purified and analysed as per Romer (1975).

Day old chicks were divided in to six groups of sixteen each. Chicks of groups I to IV received aflotoxin1 ppm per day for 2 weeks and represented as immunosuppressed chicks. The chicks of group V served as healthy vaccinated control. From 3rd to 6th week, chicks received tuftsin (10 ug/bird), stresroak (10ml/100 birds in water) and zinc sulphate (40 ppm/bird in water) as immunomodulator for groups I, II and III, respectively. On 7th day pre vaccinated titres against NDV (T-Dependent) and *S.gallinarum*(T-independent) against for all groups were estimated by micro HI and SAT, respectively. Seroconversion wasmonitored at weekly intervals by assessing specific antibody titres. Similarly, body weights were also estimated in all groups from 1st week to termination of the experiment. Finally, all the experimental chicks were challenged with both virulent ND virus and *S.gallinarum* . Statistical analysis of the data was carried out to know the significance of values observed.

Results and Discussion: Efficiency of three different immunomodulators in restoring normal immune status in immunosuppressedbroilers which were immunized with T-dependent and T-independent antigens was presented in Table1. From 3rd to 5th week, group I showed significantly higher HI titres ranging from $\log_2$ 5.2 to 7.6 which were higher than those of group II, III and IV. These results indicated total recovery from immunosuppression and restoration of normal immune response. The vaccinated control groups with aflatoxin feeding did not show significant rise in HI titre from 3rd week onwards in comparison to group 5 without aflatoxin which showed better HI titres indicating that there was no recovery from immunosuppression in group IV. SAT titres with *S.gallinarum* antigen also showed similar results. At the termination of the experiment the order of seroconversion to T-dependent antigen

was I>V>II,III>IV, while the seroconversion to T-independent antigen was I>V>II>III>IV. These results are in accordance with Sadeghi et al. (2012) who reported the similar findings while working with immunosuppressed chickens.

Body weights of broiler chicken can be considered as an indicator of feed conversion efficiency, health and immune status of the birds. The results of the presented study on the body weight gains (Table 2) clearly indicate that the aflatoxin treatment at 1ppm level for a period of 2 weeks adversely affect the bodyweight gain of chicks when compared to healthy controls (V&VI). These findings are in confirmatory with the observations of Al-Shawabkeh et al. (2009) who recorded significantly lowered weight gain in aflatoxin treated chicks.

Among these immunomodulators tested tuftsin treated group birds at the end of 6^{th} week showed improved body weight which was almost nearer to the body weight of healthy vaccinated and healthy unvaccinated control groups. In contrast, stresroak and zinc sulphate showed less effect, while group IV which received aflatoxin without any immunomodulator showed less body weights.

The results of the challenge tests with NDV (virulent virus) indicated that tuftsin, stresroak and zinc sulphate could cause immunomodulation leading to protection in immunosuppressed and vaccinated birds, and the challenge of group IV with virulent NDV showed 100 per cent protection from the development of lesions, while in unvaccinated control there was 100 per cent mortality was observed. With virulent *S.gallinarum*theses three modulators could give 100 per cent protection from development of lesions. Whereas 20 and 100 per cent of chicks showed necrotic foci on liver observed in IV and VI, respectively.

The results of the present study revealed that among the three immunomodulators, tuftsin was found to be superior followed by stresroak and zinc sulphate in restoring immune system. Tuftsin, a four amino acid peptide consisting of threonine, lysine, proline and arginine primarily stimulates macrophages and found to gear up the immune system in immunosuppressed animals (Nandinishetty, 1993).

Conclusion: Hence, it was concluded that, tuftsin proved superior as immunomodulator followed by sresroak and zinc sulphate in restoration of immune response in aflatoxin induced immunosuppression.

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Table 1: Seroconversion in experimental chicken									
Group	**NDV**					***S.gallinarum***			
	1st week**	**2nd week**	**3rd week**	**4th week**	**5th week**	**1st** to 3rd week**	**4th week**	**5th week**	**6th week**
I	2.70[b]	1.20[b]	5.20[a]	7.00[a]	7.60[a]	0	6.32[a]	7.52[a]	9.12[a]
II	2.80[b]	1.20[b]	3.10[b]	5.00[b]	5.10[b]	0	5.32[b]	5.82[b]	7.22[b]
III	2.90[b]	1.10[b]	3.10[b]	5.00[b]	5.10[b]	0	5.32[b]	5.62[b]	6.42[b]
IV	2.90[b]	1.10[b]	2.00[c]	3.00[c]	3.00[c]	0	4.32[c]	4.42[c]	5.12[c]
V	4.00[a]	4.00[a]	5.10[a]	6.00[a]	7.10[a]	0	6.32[a]	7.22[a]	8.32[a]
VI	4.00[a]	1.20[b]	0.00[d]	0.00[d]	0.00[d]	0	0	0	0

Mean values of 10 pooled samples expressed in log.
** Prevaccination titres
a,b,c= Different superscripts carrying column wise differ significantly

Table 2: Body weight gain (g) and challenge studies in experimental chicken								
Group	**1st week**	**2nd week**	**3rd week**	**4th week**	**5th week**	**6th week**	**Per cent of protection after challenge with virulent NDV**	**Per cent of birds developed specific lesions after challenge with virulent*S.gallinarum***
I	86.44[b]±0.01	191.59[b]±0.05	461.34[c]±0.74	874.08[c]±0.51	1160.47[b]±0.52	1732.68[b]±0.31	100	0
II	86.81[b]±0.21	191.23[b]±0.42	361.97[d]±0.42	744.31[d]±0.21	1039.44[c]±0.26	1415.09[c]±0.52	100	0
III	86.09[b]±0.45	191.71[b]±0.25	355.33[c]±0.61	738.42[d]±0.21	1008.21[d]±032	1277.09[d]±0.46	100	0
IV	86.79[b]±0.25	191.31[b]±0.21	293.59[f]±0.19	630.59[e]±0.40	955.40[e]±0.25	1089.10[e]±0.20	100	20(Necrotic foci on liver)
V	129.28[a]±0.25	258.56[a]±0.20	674.27[a]±0.51	1072.19[a]±0.31	1451.60[a]±0.25	1808.04[a]±0.41	100	0
VI	129.20[a]±0.11	259.10[a]±0.41	662.03[b]±0.51	1052.0[b]±0.40	1451.77[a]±0.50	1809.02[a]±0.72	0	100(Necrotic foci on liver)

a,b,c,d,e,f = Different superscripts carrying column wise differ significantly

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