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HUMORAL AND CELL MEDIATED IMMUNE RESPONSE AGAINST NDV IN TURKEY POULTS (*MELIAGRIDESGALLOPAVO* - BELTSVILLE SMALL WHITE VARIETY) FED EXPERIMENTALLY WITH AFLATOXIN AND T-2 TOXIN

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Abstract: The increased frequency of occurrence of multiple mycotoxicoses (immune-compromising entity) leads to vaccination failures which pose a great challenge in preventing Newcastle disease. Experimental mycotoxicoses were induced singly and in combination in 48 newly hatched turkey poults (*Meleagridisgallopavo* - Beltsville small white) for a period of 0 to 13 weeks by feeding diets containing 100 ppb AF and 1 ppm T-2 toxin. The AF produced on rice and T-2 toxin on corn grits were quantified by TLC Weighed amounts of powdered culture material was incorporated into the toxin free diet and were adjusted to 100 ppb AF and 1 ppm T-2 toxin. Turkey poults were vaccinated against NDV with D-58 strain by oculonasal route on the 11th day of age (primary vaccination) and 33rd day booster vaccination and the HI titres were measured at 21st day post primary and post booster vaccination to assess humoral immunity to NDV while cell mediated immunity was measured by using colorimetric blastogenesis assay in spleen during seventh and thirteenth week sacrifice. The mean HI titre to NDV decreased significantly in the toxin treated groups when compared to the control group. Among the toxin treated groups, AF-T-2 toxin treated group showed significantly higher reduction of HI titre value. These findings correlated with the lymphoid depletion and lymphocytolysis observed in the lymphoid organs of mycotoxin fed birds. A significant reduction in lymphocyte stimulation index was observed in toxin fed birds when compared to the control. Significant decrease in the lymphocyte SI was observed in AF-T-2 toxin treated groups when compared to the other mycotoxin treated groups. T-2 mycotoxin is extremely toxic to leucocytes and other rapidly dividing cells resulting in *in vivo* and *in vitro* immunosuppressing effects. Indeed, lymphocytes are more sensitive to T-2 toxin than other cell types and either DNA or protein synthesis inhibition were sensitive endpoints in cell systems when compared to general cytotoxicity. Hence, significant

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depression in the cell mediated immunity observed in this study could be due to functional impairment of splenocytes and inhibition of mitochondrial enzyme synthesis at 3 ppm T-2 toxin level. The reduction in lymphocyte stimulation index in AF-T-2 combined toxicity might be due to an additive effect of toxins on immunocytes which might result in vaccine failures and predispose the birds to secondary infections.
Keywords: Turkey poults, aflatoxin, T-2 toxin, humoral and cell mediated immune response.

INTRODUCTION

Food safety and security have generally remained basic human needs globally. Among the very many hazards contamination of food and feed by mycotoxins (toxic metabolites of fungi) in the form of multiple mycotoxicoses is the current problem faced by the poultry farmers. Aflatoxin (AF) and T-2 toxin are the most frequently encountered mycotoxins. AF is a potent hepatotoxin with dihydrofuran-coumarin moiety and is ofimportance in producing the biological effects and is produced by *Aspergillus flavus* and *A.parasiticus.* T-2 toxin is a 3 hydroxy 4, 15 diacetoxy 8 (3-methylbutryloxy), 12, 13 epoxy trichothec-9-ene metabolite. It is a potent irritant, inflammatory (dermotoxic, alimentarytoxic, hepatotoxic and growth inhibitory agent) and radiomimetic agent produced by *Fusarium* species. The AF, by binding to both RNA and DNA blocks transcription whereas, T-2 toxin blocks initiation of translation. The studies on aflatoxicosis in turkey poults were limited owing to the potential sensitivity of the species, the same on T-2 was scant and there were none on AF-T-2 combined toxicity for a period of 91days. Hence, the present study wasconducted.

MATERIALS AND METHODS

AF was produced on rice (Shotwell et al., 1966) by using A. *parasiticus* NRRL 2999 strain. The T-2 toxin was produced on corn grits (Burmeister, 1971) by using F. *sporotrichoides* MTCC 1894 strain (Microbial Type Culture Collection, Chandigarh, India). The mycotoxin content in cultured material was analysed at Pharmaco vigilance Laboratoryfor Animal Feed and Food Safety (PLAFFS), Centre for Animal Health Studies, TANUVAS, Madhavaram Milk Colony, Chennai, Tamilnadu, India. Known amounts of AF and T-2 toxin containing powdered substrates were incorporated into turkey brooder mash both singly and in combination to yield 100 ppb AF and 1 ppm T-2 toxin. Fortyeight newly hatched unsexed turkey poults obtained from standard hatcheries were wing banded, weighed and housed in battery brooders with ad libidum supply of feed and water. They were randomly distributedinto four groups of twelve chicks each. The control and toxin mixed diets were fed todifferent groups for 91 days from the day of hatch. Newcastle disease virus (NDV) D-58 strain of live thermostabilized vaccine used in the trial was procured from the Department of

Veterinary Microbiology, Madras Veterinary College, Chennai. Turkey poults were vaccinated against NDV with D-58 strain by oculonasal route on the 11th day of age (primary vaccination) and 33rd day booster vaccination and the HI titres were measured at 21st day post primary and post booster vaccination. The haemagglutination inhibition test was done at the Department of Veterinary Microbiology, Madras Veterinary College to find out the HI titre to NDV. Cell mediated immunity to NDV was measured by using colorimetric blastogenesis assay in spleen during seventh and thirteenth week sacrifice as described by Reynolds and Maraqa (2000). The blastogenic responses for the MTT assay were expressed as a mean stimulation indices (SI) by dividing mean absorbance of stimulated culture (Cs) minus mean absorbance of unstimulated culture (Cu) by mean absorbance of unstimulated culture.

$$SI_{MTT} = (C_s - C_u) / C_u)$$

RESULTS

Humoral immunity

Mean (± SE) HI titre to NDV in turkey poults fed AF and T-2 toxin singly and in combination are presented in Table 1. Comparison of overall means revealed significant ($P<0.05$) differences between the control and mycotoxin treated groups. The AF-T-2 group differed significantly ($P<0.05$) for other groups. Significant ($P<0.05$) decrease in the HI titre to NDV was observed in the toxin treated group when compared to the control group.

Cell mediated immunity

Mean (± SE) lymphocyte stimulation indices (SI) in turkey poults fed AF and T-2 toxin singly and in combination are presented in Table 2. Comparison of overall means revealed significant ($P<0.05$) differences between the control and mycotoxin treated groups. The AF-T-2 group differed significantly ($P<0.05$) from other toxin fed groups. Significant ($P<0.05$) decrease in the lymphocyte SI was observed in the toxin treated group when compared to the control group.

DISCUSSION

Humoral immunity

The mean HI titre to NDV decreased significantly in the toxin treated groups when compared to the control group. Among the toxin treated groups, AF-T-2 toxin treated group showed significantly higher reduction of HI titre value. The findings concurred with earlier findings in White Leghorn chicks fed AF 0.2 ppm and above (Mangat *et al*., 1989; Azzam and Gabal, 1998), broiler chicks fed T-2 toxin 0.5 ppm and above (Kamalavenkatesh, 2003; Krishnamoorthy, 2004). These findings correlated with the lymphoid depletion and

lymphocytolysis observed in the lymphoid organs of mycotoxin fed birds. Sklan *et al*. (2003) did not observe reduction in serum titre of turkey poults fed 1 ppm T-2 toxin for 32 days. This might be due to the inherent ability of the T-2 toxin in inhibiting the biosynthesis of proteins and other macromolecules by binding to the 60s ribosomal subunit and inhibitory interaction with the enzyme peptidyl transferase (Bunner and Morris, 1988; Corrier, 1991). These findings correlated with lymphoid depletion observed in lymphoid organs in histopathological findings in this study.Further the alteration in immunocompetence of toxin treated birds may also be due to impaired function of B & T cells and protein synthesis which could adversely affect the humoral immunity in toxin treated groups (Wyatt, 1991).

Cell mediated immunity

A significant reduction in lymphocyte stimulation index was observed in toxin fed birds when compared to the control. Significant decrease in the lymphocyte SI was observed in AF-T-2 toxin treated groups when compared to the other mycotoxin treated groups. The findings are in accordance with Babu Prasath (2008) who observed a significant decrease in the lymphocyte SI for 1 and 3 ppm T-2 toxin fed birds.

T-2 mycotoxin is extremely toxic to leucocytes and other rapidly dividing cells resulting in *in vivo* and *in vitro* immunosuppressing effects. Indeed, lymphocytes are more sensitive to T-2 toxin than other cell types and either DNA or protein synthesis inhibition were sensitive endpoints in cell systems when compared to general cytotoxicity (Forsell *et al*., 1985) and therefore the capacity of the mitochondrial enzyme succinate dehydrogenase to transform the tetrazolium salt of MTT into blue coloured formazan is inhibited. Hence, significant depression in the cell mediated immunity observed in this study could be due to functional impairment of splenocytes and inhibition of mitochondrial enzyme synthesis at 3 ppm T-2 toxin level. However, significant reduction observed in the AF fed birds in the SI values of spleen concurred with the findings of Gounalan (2005) who fed 0.5 ppm to layer chicks for 12 weeks and Ajith Jacob George (2007) who fed 50, 150 and 300 ppb AF in broiler chicken from 0 to 42 days. Leeson *et al*. (1995) opined that in aflatoxicosis cell-mediated immune response and effector cell function were affected. Wyatt (1991) postulated that AF interfered with normal function of B- and T-cells, rather than causing destruction of these cells. The impairment of protein synthesis caused by dietary AF could account for the lack of humoral immunity without the necessity of B- and T-cell destruction. Regardless of the atrophy of the bursa of Fabricius and thymus gland, the apparent alteration of splenic function was also of

diagnostic significance and implied alteration in the immunocompetence of birds with aflatoxicosis (Richard *et al*., 1975).

No comparable literatures on reduction in lymphocyte stimulation index in AF-T-2 combined toxicity was available for turkey poults. However, significant decrease in lymphocyte stimulation index observed among other mycotoxin treated groups might be due to an additive effect of toxins on immunocytes which might result in vaccine failures and predispose the birds to secondary infections.

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TABLE 1
Mean (±SE) HI titres against NDV in turkey poults fed aflatoxin and T-2 toxin singly and in combination

(n=6)

Groups	MDA HI titre (log^2)	Age in weeks HI titre (log^2)		Overall means
		Primary	Booster	
Control	5.33±0.82	4.17 ±0.32	4.83±4.10	4.50^a±0.20
Aflatoxin (100 ppb)	5.16±0.75	3.08±0.22	3.17±2.69	3.13^b±0.15
T-2 toxin (1 ppm)	5.50±0.84	2.92±0.23	2.83±2.41	2.88^b±0.16
Aflatoxin (100 ppb) + T-2 toxin (1 ppm)	5.33±0.82	2.33±0.23	2.17±1.84	2.25^c±0.15

Overall means bearing same superscripts within a column do not differ from each other (P>0.05)

TABLE 2

Mean (±SE) stimulation indices of splenocytes in turkey poults fed aflatoxin and T-2 toxin singly and in combination

(n=6)

Groups	Stimulation indices of splenocytes		Overall means
	7^{th} week	13^{th} week	
Control	0.34±0.05	0.31±0.04	0.32^a±0.05
Aflatoxin (100 ppb)	0.20±0.09	0.19±0.04	0.20^b±0.07
T-2 toxin (1 ppm)	0.20±0.09	0.14±0.03	0.16^b±0.07
Aflatoxin (100 ppb) + T-2 toxin (1 ppm)	0.13±0.05	0.08±0.02	0.10^c±0.04

Overall means bearing same superscripts within a column do not differ from each other (P>0.05)