

IMMUNOMODULATORY EFFECT OF SUBLETHAL DOSE OF CITRININ AND AFLATOXIN IN BROILER CHICKEN*

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ABSTRACT

The present study was planned to find out the individual and combined immunomodulatory effects of sublethal doses of citrinin (CTN) (5 ppm) and aflatoxin (AF) (0.5 ppm) in broiler chicken. Ninety-six newly hatched broiler chicks were randomly allotted to 4 groups of 24 birds each and fed with control, CTN (5 ppm), AF (0.5 ppm) and CTN (5 ppm) + AF (0.5 ppm) diets from 0 to 6 weeks of age. The birds were vaccinated against Newcastle disease (ND) virus on 7th and 28th day of age. The HI titres to ND vaccination and the stimulation index of splenocytes decreased significantly ($P < 0.05$) in all the mycotoxin treated groups when compared to the control group. The present study indicated that CTN even at the sublethal dose level could lower the immune status of the birds and among the mycotoxin fed groups. AF played a more potential role in causing the reduction when compared to CTN.

Key words: Citrinin, aflatoxin, broiler chick, HI titres to ND, stimulation index, immunity.

INTRODUCTION

The fungi that grow on a variety of feedstuffs and foods consumed by animals and man are ubiquitous in Indian agroclimatic conditions. Mycotoxins are structurally diverse secondary metabolites of fungi, which cause mycotoxicosis in single or mycotoxicoses in mixed conditions. Citrinin (CTN) and aflatoxin (AF) are two fungal secondary metabolites that affect the immune status and thereby the productivity of broiler chicken. At present, CTN is seen together with AF more frequently in the feed samples. The previous studies on CTN toxicity in broiler chicken was conducted using CTN from 100 ppm onwards, but

in this study only 1/50th of the dose of CTN was used in chicken to find out the difference. Considering the increased frequency of co-occurrence of CTN and AF in the feed at sublethal levels and the paucity of literature on the immunomodulatory effect of CTN and AF at sublethal doses in broiler chicken, this study was taken up

MATERIALS AND METHODS

Citrinin was produced using *Penicillium citrinum* NRRL 5927 culture (Carlton *et al.*, 1974) on maize (Nelson *et al.*, 1980) and rice (Carlton *et al.*, 1974) and the AF was produced

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*Forms part of the Ph.D. thesis of the first author approved by the Tamil Nadu Veterinary and Animal Sciences University, Chennai -600051.

using *Aspergillus parasiticus* NRRL 2999 on rice (Shotwell *et al.*, 1966). Two experimental trials were conducted using 96 broiler chicks. In each trial, a total of 48 newly hatched broiler chicks were randomly allotted to 4 groups of 12 birds each and fed with control, CTN (5 ppm), AF (0.5 ppm) and CTN (5 ppm) + AF (0.5 ppm) diets from 0 to 6 weeks of age. Six birds from each group were sacrificed at 3rd and 6th week of age.

ND vaccination schedule: The birds were vaccinated against ND with NDV-B₁ at a dose rate of 10⁶ EID₅₀/bird and NDV-La Sota at the dose rate of 10⁶ EID₅₀/bird by oculo-nasal route on 7th and 28th day of age, respectively.

Humoral immunity to ND: The haemagglutination inhibition (HI) titres against NDV were measured both in the control and mycotoxin treated birds at third and sixth weeks of age (OIE, 2004).

Cell mediated immunity to ND: To analyze the functional characteristics of lymphocytes, lymphocyte proliferation assay was carried out using splenocytes with Con A (Sigma, USA) as a mitogen. Lymphocytes were prepared from spleen of control and toxin treated birds (three birds from each group) on 6th week of age (Wu *et al.*, 2000). The MTT (3-[4-5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay for proliferation of splenocytes against Con A was essentially that described by Bounous *et al.* (1992) with some modifications. The formazan crystals formed after 4 h incubation of cells with MTT (Sigma, USA) were dissolved by adding 150 µl of dimethyl sulphoxide (Sigma, USA) to each well instead of isopropanol acid (0.04 N HCl) since better solubilization of the formazan crystals was achieved by dimethyl sulphoxide and these solubilized crystals gave a greater optical density (Twentyman and Luscombe, 1987). The mean optical density was read on an ELISA reader (µ Quant-Biotec instruments Inc., USA).

Blastogenic responses for MTT assay were expressed as a mean stimulation index (SI) by dividing OD values of stimulated cells (Cs) minus OD values of unstimulated cells (Cu) by OD values of unstimulated cells. $SI_{MTT} = (Cs-Cu)/Cu$. The data generated were subjected to two way analysis of variance using SPSS version 9.0 software for windows.

RESULTS AND DISCUSSION

Penicillium citrinum NRRL 5907 culture material yielded 320 ppm CTN in maize and 187 ppm CTN in rice. The *Aspergillus parasiticus* NRRL 2999 rice culture material yielded 112 ppm AF. In the entire toxin fed groups, inappetance and brownish diarrhoea were observed from first week onwards. The AF and CTN+AF fed groups showed ruffled feathers, lethargy, and stunted growth from third week. In the CTN+AF group the clinical signs were more pronounced. No mortality was observed in the control and mycotoxin fed groups.

i) Humoral immunity: Mean ± SE HI titres to NDV in broiler chicks fed with control, CTN, AF and CTN+AF diets are shown in Table 1. The overall means for the ND-HI titres were 86.75 ± 18.22, 52.25 ± 12.14, 39.75 ± 11.98 and 35.00 ± 9.67 for control, CTN, AF and CTN+AF groups, respectively. The HI titres against NDV decreased significantly (P<0.05) in all the mycotoxin treated groups when compared to the control group. In the present study, apart from hypoglobulinaemia, histopathological lesions revealed lymphoid depletion in the bursa of Fabricius, spleen, caecal tonsils and Harderian gland in the mycotoxin fed groups, which might have had the deleterious effect on the humoral immunity of the birds. The AF group showed significant reduction in the ND-HI titre values, which indicated significant reduction in the humoral immunity. Similar findings were reported in broiler chicks fed from 0.1 ppm AF for five weeks (Devurkar *et al.*, 1995), eight weeks (Mani *et al.*, 2000), 20 days (Gupta *et al.*, 2003) and 12 weeks in layer chicks (Gounalan,

2005). Devurkar *et al.* (1995) reported a marginal reduction at 0.125ppm AF and a four-fold decrease in HI titre level to ND at 0.5 ppm AF. However, no significant changes in ND-HI titre were observed in broiler chicks fed 100, 200, 400, 500 and 1000 ppb AF from 2 to 7 weeks of age (Giambrone *et al.*, 1985) and 70 ppb AF from 42 days (Perozo and Rivera, 2003). Among the mycotoxin fed groups, there was no significant difference but comparison of the numerical values between the toxins fed groups indicates that AF plays more potential role in causing the reduction when compared to CTN. No comparable literature was available for CTN+AF group. These findings correlated with the lymphoid depletion and atrophy in the lymphoid organs, decrease in the stimulation index of splenocytes, hypoproteinaemia in the mycotoxin fed birds that could have contributed to the lower HI titre to ND

ii) Cell mediated immunity: Mean \pm SE stimulation index (SI) in broiler chicks fed with control, CTN, AF and CTN+AF diets were 0.870 ± 0.020 , 0.400 ± 0.060 , 0.100 ± 0.017 and 0.090 ± 0.040 for the control, CTN, AF and CTN+AF groups, respectively (Table 1). The SI index of splenocytes to con A decreased significantly ($P < 0.05$) in all the mycotoxin treated groups when compared to the control group. Though in CTN group a significant reduction in stimulation index was not observed when compared to AF and CTN+AF groups the present study indicates that CTN level even at the sub lethal dose of 5 ppm could lower the immune status of the birds. The immunomodulatory effect of CTN at 5ppm level in broiler chicks concurred with the findings of Kumar *et al.* (2014) who found a peak induction of apoptosis of splenic lymphocytes after 48 hours (h) of feeding 5ppm CTN in 3 week old broiler chicks. Tammer *et al.* (2007) observed a decreased production of IFN- γ and suppression of cytokine production in a concentration-dependent manner when stimulated human peripheral blood mononuclear cells were exposed to CTN.

In the AF fed birds, significant reduction observed in the SI values of spleen concurred with the findings of Gounalan *et al.* (2006) who fed 0.5 ppm to layer chicks for 12 weeks. The CTN+AF fed birds showed significant reduction in SI of spleen that was comparable to that of AF group. Hence, AF is more potential than that of CTN in affecting the cell mediated immunity. The immunosuppressive effect of AF and CTN+AF concurred with the findings of Kumar *et al.* (2014) who found a peak induction of apoptosis of splenic lymphocytes after 24h post feeding for AF and CTN+AF and peak induction of necrosis of splenic lymphocytes after 48h post feeding of CTN+AF in 3 week old broiler chicks. To conclude, the present study also revealed that the mycotoxicoses caused severe hepato-renal and gastro-intestinal tract damage with a resultant hypoproteinaemia and hypoglobulinemia which might have further lead to immunocompromisation in the mycotoxin fed birds.

REFERENCES

- Bounous, D.I., R.P. Campagnoli and J. Brown. 1992. Comparison of MTT colorimetric assay and tritiated thymidine uptake for lymphocyte proliferation assays using chicken splenocytes. *Avian Diseases*, **36**: 1022-1027.
- Carlton, W.W., G. Sansing, G.M. Szczech and J. Tuite. 1974. Citrinin mycotoxicosis in beagle dogs. *Food and Cosmetic Toxicology*, **12**: 479-490.
- Devurkar, U., G. Devegowda and K. Prabhudas. 1995. Immunosuppression during aflatoxicosis in broiler chicken. *Indian Journal of Poultry Science*, **30(3)**: 255-256.
- Giambrone, J.J, U.L. Diener, N.D. Davis, V.S. Panangala and F.J. Hoerr. 1985. Effects of aflatoxin on young turkeys and broiler chickens. *Poultry Science*, **64(9)**: 1678-84.

- Gounalan, S. 2005. Pathology of Newcastle disease virus and its interaction with mycotoxins in layer-type chicken. Ph.D., thesis approved by Tamil Nadu Veterinary and Animal Sciences University, Chennai.
- Gounalan, S., C. Balachandran and B. Murali Manohar. 2006. Immunopathological effects and induction of apoptosis in spleen and thymus of layer chicken in aflatoxicosis. International Conference on Advanced Veterinary Practice in Medicine and Surgery – Augmenting Health and Production, 21-25, June, Chennai, Compendium of Lead Papers and abstracts, p. 65.
- Gupta, K., Ram Neek and A. Singh. 2003. Immunomodulatory effects of aflatoxicosis, infectious bursal disease and their interaction against Newcastle disease vaccination in broilers. *Indian Veterinary Journal*, **80**: 78-80.
- Kumar, C.T.A and Balachandran, C. 2014. Detection of apoptosis in citrinin, aflatoxin and their combined effects in broiler chicken. *Indian J Vet. Pathol.*, **38 (4)**: 256-60.
- Nelson, T.S., J.N. Beasley, L.K. Kirby, Z.B. Johnson and G.C. Ballam. 1980. Isolation and identification of citrinin produced by *Penicillium lanosum*. *Poultry Science*, **59**: 2055-2059.
- OIE (Office International des Epizooties). 2004. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees), 5th Edn, Vol. I, France.
- Perozo, F and S. Rivera. 2003. Effect of aflatoxin B₁ exposure and selenium supplementation on immune response in broilers. *Indian Veterinary Journal*, **80**: 1218-1221.
- Shotwell, O.L., C.W. Hesseltine, R.D. Stubblefield and W.G. Sorenson. 1966. Production of aflatoxin on rice. *Applied Microbiology*, **14(3)**: 425 - 428.
- Tammer B, Lehmann I, Nieber K, Altenburger R. (2007). Combined effects of mycotoxin mixtures on human T cell function. *Toxicology Letters*. **170(2)**:124-33.
- Twentyman, P.R. and M. Luscombe. 1987. A study of some variables in a tetrazolium dye (MTT) based assay for cell growth and chemosensitivity. *British Journal of Cancer*, **56 (3)**: 279-285.
- Wu, C.C., T. Dorairajan and T.L. Lin. 2000. Effect of Ascorbic acid supplementation on the immune response of chickens vaccinated and challenged with infectious bursal disease virus. *Veterinary Immunology and Immunopathology*, **74**: 145-152.

Table 1

Mean (\pm SE) ND-HI titres and stimulation index of splenocytes to concanavalin A in broiler chicken fed with control, CTN, AF and CTN+AF diets

Groups	ND-HI titres (n=12)			Stimulation index of splenocytes to concanavalin A (n=3)		
	3 rd week	6 th week	Overall means	Unstimulated	Stimulated	Stimulation index
Control	52.17 \pm 14.40	121.33 \pm 31.03	86.75^a \pm 18.22	0.527 \pm 0.0720	0.874 \pm 0.073	0.870^a \pm 0.020
CTN (5 ppm)	42.17 \pm 13.06	62.33 \pm 20.66	52.25^b \pm 12.14	0.554 \pm 0.0850	0.768 \pm 0.089	0.400^b \pm 0.060
AF (0.5 ppm)	29.50 \pm 10.43	50.00 \pm 21.72	39.75^b \pm 11.98	0.543 \pm 0.730	0.658 \pm 0.053	0.100^c \pm 0.017
CTN + AF	26.17 \pm 11.52	43.83 \pm 15.64	35.00^b \pm 09.67	0.398 \pm 0.770	0.440 \pm 0.069	0.090^c \pm 0.040

Geometric mean HI titres expressed as reciprocal log₂

Overall means and means with different superscripts differ significantly (P<0.05)